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A STUDY IN CONNECTION WITH THE PROBLEM OF HORMONIZATION OF SEEDS

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CONTENTS

List of abbreviations and commercial preparations

c.p.a. — p-chlorophenoxyacetic acid
2,4-D — 2,4-dichlorophenoxyacetic acid
2,4-DK — potassium salt of 2,4-D
2,4-DNH_4 — NH_4 salt of 2,4-D
e.i. — 3-ethylindole
i.a.a. — indole-3-acetic acid
i.a.a.K — potassium salt of i.a.a.
i.a.a.Na — sodium salt of i.a.a.
i.a.d. — indole-3-acetamide
i.b.a. — indole-3-butyric acid
i.b.a.K — potassium salt of i.b.a.
i.m.m.a. — (indole-3-methyl)-malonic acid
i.p.a. — indole-3-propionic acid
n.a.a. — naphthalene-1-acetic acid
n.a.a.K — potassium salt of n.a.a.
n.a.a.Na — sodium salt of n.a.a.

n.a.d.	—	naphthalene-1-acetamide
n.o.a.a.	—	2-naphthoxyacetic acid
ph.a.a.	—	phenyl acetic acid
sol.	—	solution
t.i.b.a.	—	2,3,5-triiodobenzoic acid
t.u.	—	thiourea
vit.	—	vitamin

Agrosan	—	a seed disinfectant containing an organic mercury compound
Auxan	—	commercial powder preparation containing a growth substance
Auxilin	—	commercial preparation containing i.b.a. (Pennsylvania Chemical Co)
Belvitan	—	commercial preparation containing i.a.a.+i.b.a. (I.G. Farben Ind., Bayer-Leverkusen)
Ceresan	—	a powder disinfectant containing ethyl mercury phosphate
Euradin	—	commercial preparation containing n.a.a.K
Germisan	—	a seed disinfectant containing an organic mercury compound
Granosan	—	a proprietary mercurial seed dressing manufactured by the Bayer Semesan Co in the U.S.A.
Hormodin	—	commercial preparation containing i.b.a. (Merck Co)
Merck dust	—	commercial powder preparation containing i.b.a. (Merck Co)
Roche 202	—	commercial preparation containing n.a.a. (Hoffmann – La Roche)
Rootone	—	commercial powder preparation containing n.a.a. (American Chem. Paint Co)
Semesan	—	a powder disinfectant containing 30% hydroxymercurichlorophenol
Spergon	—	a seed disinfectant containing tetrachloro-p.quinone

INTRODUCTION

Statement of the problem

The idea of stimulating the germination of seeds by a particular treatment which might improve growth, development and crop as well, evidently dates from long ago. The ancients sought to realize it by the use of manure and urine.

After it became known that simple chemical compounds could act as fertilizers they soon found employment in the treatment of agricultural seeds. About 1760 the increasing knowledge of plant-diseases and their control led to the use of copper salts and mercury salts for seed disinfection.

In a modern sense both processes survive in the form of the so-called seed-dressing: the coating of seed with a combination of nutrients and disinfectants, in order to protect it against attacks by micro-organisms, fungi and insects.

Under the influence of the hormone-theory, conviction gained ground that in plants development is regulated by specific substances from the very beginning. It was found that the endosperm of different kinds of seeds contained one or more hormone-like substances, taken up by the embryo in the initial stages of development, and that subsequent processes were closely interrelated with those occurring during the first stages (149, 171, 178, 182).

So it was obvious to suppose that a lack of germinative power might be due to a shortage of natural auxin, which, in the first place, might arise from prolonged storage, causing inactivation. A possible interference by phytohormones with the processes resulting from the so-called vernalization was also taken into account.

In short, when the time was ripe for it attempts were made to influence the germination of seed and the development of the plant by administration of *synthetic growth substances.*

CHOLODNY, in 1936 (25, 26), was the first to report positive results from seed-treatment with hetero-auxin. From then research in this domain expanded rapidly, until, since 1944, interest waned more and more, as appears from the number of papers on this subject (table I).

TABLE I

Number of papers concerning the treatment of seeds with growth substances during 1936—1951.

Year	Number	Year	Number
1936	3	1944	5
1937	5	1945	3
1938	12	1946	2
1939	9	1947	5
1940	14	1948	6
1941	18	1949	3
1942	20	1950	2
1943	13	1951	1

In the author's opinion this was occasioned by manifold discrepancies in the results. A further explanation is offered in chapter I.

Together with VELDSTRA (115) it may certainly be concluded that in the matter of seed-treatment the application of plant growth substances stands a good chance.

However, before arriving at applications useful in practice, the many factors ruling the processes of germination and development will have to be appreciated.

Therefore the present study was initiated with an analysis of the influence of exogenous factors upon the development of the embryo. The results may tend to obtain some more insight into the correlation-phenomena, playing a part during germination under normal conditions, and their influencing by treatments of the seed with growth substances.

CHAPTER I

SURVEY OF THE LITERATURE

(Treatment of seeds with synthetic growth substances)

Various effects have been pursued in the treatment of seeds with growth substances, mainly in view of practical applications.

In the first place it was tried *to improve the germination* of aged or badly germinating seeds, or *to arrive at larger crops* by influencing subsequent development (7). For, a particular treatment of the seed may raise not only the percentage of *germination* but its *rate* as well (8). An advance like this, as compared with non-treated material, conceivably may subsist down to harvesting. These differences, however, usually vanish during development (52, 64, 121).

In certain circumstances an *acceleration of growth* was observed (23) and, probably owing to this and to an *enlarged root-system* (9, 53), also an *advanced flowering* in comparison with the controls (107, 110, 113).

Breaking the natural dormancy of the seed was reported in a few cases only (14, 100). More recently, the deleterious effects of 2,4-dichlorophenoxyacetic acid and other herbicides have been used to advantage in preventing the germination or in killing the very young seedlings of some weeds. This phase of growth substance effects on germination seems to have much more promise of successful application (122–140, 230).

A phenomenon, stated first of all by GRACE in 1938, looked promising, viz. that the *injury inflicted upon seeds* by fungicides, hot water or other seed disinfectants *might be reduced* or even abolished by means of growth substances (32, 58–62, 116).

Finally it may be mentioned that after treating seeds with growth substances the plants showed *more resistance to diseases* (43, 90, 96).

Unlike other practical applications of growth substances in which the number of methods is but very limited, in seed treatment they widely diverge. Considering the large diversity of objects treated and the varying conditions, it is not to be wondered at that results were often contradictory.

A lengthy discussion of the papers on the subject would involve a great many of details and is intentionally omitted. However, to meet the convenience of those who want to consult the literature and for the purpose of surveying the results obtained so far, a number of data is summed up in table II.

Enumeration of both positive and negative results from the table shows that treatment with growth substances favoured *germination* in 81 cases, whereas an unfavourable effect or none at all was seen in 169 instances. Consequences on further *development* appeared to be positive in 115 cases; there were 104 negative effects.

Summarizing, in the treatment of seeds by means of growth substances negative results are predominant. It certainly will be more conspicuous on closer examination of the data.

Now it may be considered what errors have been made in research and from what sources the numerous, conflicting statements may have sprung.

1. *General experimental technique.* Common shortcomings are: the omission of replications (16, 17, 25), experiments on a scale too small to judge of (24, 25, 107–110) and the lack of control groups, the seeds

of which are sown in the dry state, without any treatment (e.g. 18, 22, 23, 25, 26, 74, 96, 110). Many research-workers have made these mistakes, thus rendering their results anything but reliable. If, owing to some treatment, part of the objects should die off they might procure thus much space in the trial-plot that the remaining plants ultimately would produce a larger yield (42, 49, 51, 55, 105). A wrong interpretation would class such a case among the favourable results obtained with growth substances.

2. *Influences of the object itself.* The effect of growth substance treatment will be highly dependent on the fact, whether or not the plant is able to supply its want of hormones by synthesis. In the former case no result is to be expected, whereas the latter does offer a possibility of overcoming a hormone deficiency (52, 68, 112, 117, 121).

It may be expected, therefore, that certain influences, exerted upon the mother-plant, will continue to take effect via the seed. For example, the seeds harvested after a period of drought, mainly being tiny, will raise a progeny less vigourous than that of larger seeds, formed under better growth conditions.

Though it has scarcely been touched upon in the literature, it may be assumed that some part is played by the nature and by the amount of reserve substances and ergons (i.e. the proportion embryo-size/mass of reserve substance). Reference to this matter is found under 67 and 95. Moreover, there may be influences of *origin* (10), *age* (9, 10, 86, 103), *dormancy*, *after-ripening*, *method of storage*, *differences in the permeability of the seed-coat*, and *inhibitory substances*, being present or otherwise (21, 40).

It would seem that the diversity within a given lot of seed is not properly realized, although several papers have pointed out the interest of *physiological condition* (52), *size* and *weight* of the seed with respect to the resulting crop (200-212). In multispermous fruits differences occur between the component seeds (100).

Many seeds are more or less injured by mechanical threshing. It is conceivable that the effect of a growth substance may be different, as it enters through the seed-coat or by way of a damaged spot. Besides, it is well-known that various, badly germinating seeds may develop well, after the seed-coat has been injured, either mechanically or chemically. This usually means an aid in clearing away a mechanical resistance offered by the seed-coat in germination.

All these points present possibilities of explaining, for instance, the contradictions arising between experiments on a small scale, under well-controlled conditions, and those on a larger scale, made in practice.

The difference in behaviour between varieties and strains with respect to similar treatment was pointed out already by McRostie, Hopkins and Grace (80); it was found again in later experiments (32, 42, 47, 52, 97, 103). Early strains generally would require a concentration of the growth substance lower than that, desirable for late strains (94, 97).

3. *Choice of the growth substance, method employed and manner of*

treatment. As mentioned already at the beginning of the chapter, there are almost as many methods as there are authors; standardization is out of the question.

a. Choice of the growth substance. As a matter of course this choice mostly will determine the ultimate result. It would appear that an injurious side-effect of certain growth substances sometimes may be counteracted by adding thiourea or a combination of it with vitamin C (4, 5, 6, 94, 96). This is contested by others (51), who ascribe it to an effect of pH alone. Some investigators prefer certain salts (8, 86) or a combination of several growth substances (8, 49, 103). A minor contamination of the substance may influence its effect to no small degree (61).

b. Method of administration. Roughly speaking two methods should be distinguished: a *dust-treatment* (growth substance mixed with powdered talcum, charcoal (96) or a seed disinfectant) and the method of *soaking* in a solution, which is subject to two variations: a *superficial spraying* (44, 73, 98) or a *brief submersion* (85).

On this point a good deal of variation is found in the literature, e.g., preliminary germination in water is applied before the growth substance is added (21); the seeds are continuously exposed to the growth substance by allowing them to germinate on a medium containing the active compound (36, 82, 102); after 24 hours' soaking in a solution of growth substance the seeds are rinsed with water (7); after soaking in the solution sowing is done either direct or after the seeds have been rinsed with water and allowed to dry (6).

Too little attention has been paid to the fact that many kinds of seed are apt to meet with 'soaking injury', when soaked in solutions (190–199). That the oxygen content of the solution does affect the growth of oat coleoptiles after the treatment has been made admissible by Albaum, Kaiser and Eichel (1).

The leaching of inhibitory substances during wet treatment may be another cause of varying results (21, 40). In addition, it is imaginable that, by soaking in solutions, seeds will lose part of their own growth hormones.

In general the seeds are sown immediately after treatment. Subsequent storage in the dry state would give less favourable results (47, 91).

The usual methods of applying the growth substance in solution, even at a low concentration, in many cases do retard root growth. So a dust treatment is recommended by Grace and co-workers, as in this way a gradual supply of active material is realized.

c. Duration of treatment and concentration. It must be emphasized that the concentrations, optimal for germination, are quite different from those required in further growth. In normal development of a plant the dosage of natural hormones is a matter of careful regulation owing to correlation. The treatment of seed, however, resembles an 'initial loading therapy' which, obviously, in many cases will not completely fulfil the requirements of the material at the moment.

The *influence of the concentration* is of importance and accordingly has been studied in detail by various authors (50, 117). Even a slight

over-dosage of growth substance may prove harmful; the same applies to traces of alcohol contained in the solvent (27).

A non-sterile technique, attuned to practical application, involves the chance of destruction or additional formation of growth substances by the action of bacteria, thus causing uncertainty about the concentration. So positive results, once obtained, probably must not be generalized without comment in order to arrive at a definite instruction for use, the hormone metabolism of the seed playing a part as well.

Growth substances in the form of salts, notably at high concentrations, are alleged to be less poisonous (8). PODEŠVA (96), however, states that particularly at high concentrations potassium salts are more deleterious than free acids.

Reports about the *duration of the treatment* widely differ. If it is a lengthy one, the above-mentioned factors of soaking injury and leaching of inhibitors assert themselves. Here too, it is impossible to generalize, as every type of seed behaves differently.

d. Temperature. As a rule no attention is paid to the temperature of the growth substance solutions, only a few data being recorded. DYKYJ and DYKYJ-SAJFERTOVÁ (50), and PODEŠVA (97) afterwards, found that the penetration of growth substances into the seed is promoted by rise of temperature.

e. Acidity. DYKYJ and DYKYJ-SAJFERTOVÁ not only studied the effect of the temperature but that of the pH as well, principally with a view to applying the seed-treatment in sugar-beet culture (47, 51). CHADWICK and SWARTLEY (24) likewise have drawn attention to this subject.

Low pH and high temperature of a solution may give rise to over-dosage (97). The use of tap water as a solvent is not recommandable either, since it is usually too hard (47, 51).

As the dissociation of acidic growth substances is determined by the pH of their solutions, the growth hormone effect will depend on the pH as well. Some workers, therefore, apply a growth substance together with a buffer (39).

f. The influence of light during treatment. Although there is a possibility of certain seeds being liable to the action of light, no data on this subject are to be found.

4. *Condition during germination.* Different species of seeds certainly must not be regarded from the same point of view, which is already adequately demonstrated in Nature by the wide variation existing in biochemical processes during germination. Thus, HSUEH and LOU (67) have pointed out the completely different effect of the growth substance upon seeds, germinating either under aerobic or under anaerobic conditions.

It is not at all imaginary that some mutual influencing may arise from the excretion of inhibitory substances if, for instance, in laboratory germination tests the seeds are laid down too closely (cf. FRÖSCHEL).

LAFFERTY (73) pointed out that especially the humidity of the medium plays an important part in germination.

5. *Significance of the circumstances during culture.* From various

quarters already attention has been directed to the influence of the *season* (9, 10, 120) and of the *climate* in general (2, 5, 32, 42, 48, 51, 53, 54, 97, 103).

Söding and co-workers (103) found differences between *sunny* and *shady* plots (see also 74), whereas it appeared that in experiments like these the *alkalinity of the spraying water* deserves attention too (24).

Of course the *influence of the soil* is a matter of weight. The *temperature of the soil* (47), as well as the *humidity* (2, 5, 46, 66, 73, 92), the *pH* (93) and the *humus content* (93, 120) separately may exert an influence upon the result of a treatment with growth substances. In a cold and moist soil, for example, some authors established a diminishing of the toxic effect of certain treatments (47).

Furthermore, the *condition of the soil* plays a part (2, 5, 10, 31, 34, 42, 47, 48, 51–54, 90, 103, 106, 112, 117), and so do the *manuring* (90, 106, 107, 111, 112) and the *tillage* (42, 48). An abundant occurrence of nutrients in the soil may mask the stimulative effect of growth substance treatment (73, 103, 106, 112). A positive outcome was obtained only on normal supply of nitrogen; neither omission nor excessive administration of nitrogen led to any result (5, 68).

All these factors affecting the treatment with growth substances, several workers have arrived at the conclusion that *results are to be expected only under average growth circumstances* (2, 66, 91, 92, 96, 97, 103).

6. *Consequences in the event of a culture not being adapted to the demands of a treatment with growth substances.* Friedrich (54) has called attention to the fact that the treatment in question may inhibit development, thus causing retarded harvesting, which may involve the risk of the seeds and seedlings becoming attacked by parasites. Preliminary experiments in the hothouse often turn out well, owing to adequate supervision and nurture, while in the field failures are no exception. On account of their retarded development the objects treated will have to be reaped later than the controls and, probably, really will afford positive results, if only the grower does not cling to traditional methods.

Considering the above, it becomes acceptable that various authors advocate the desirability of making small-scale experiments beforehand (103, 117). Apart from the difficulties attended with it, this method does not afford the solution of the main problem, namely, *the explanation of the numerous contradictions in the matter of seed-treatment with growth substances.*

In the present case a prosecution of the investigations will profit greatly by strictly standardizing the circumstances of the experimental technique. This will require carefully selected material, the properties of which are known as fully as possible.

When sufficient knowledge has been gathered about the hormone metabolism of the seed and about the mechanism of germination (after administration of growth substances or otherwise), the connection between these processes and the many variable, external factors will have to be elucidated systematically. Only then one may expect this complicated matter to yield important results in practice.

TABLE II

Data on the treatment of seeds with synthetic growth substances

Species	Treatment [1])	Results [2]) 1. in germination	Results [2]) 2. in development and yield	References
CONIFERAE				
Chamaecyparis lawsoniana	i.b.a.	+		120
Ginkgo biloba *)	i.a.a. or n.a.d. mixed with talc powder	—		24, 109
Juniperus procumbens *)	i.a.a. or n.a.d. mixed with talc powder	—		24, 109
Larix decidua	Roche 202	+?	+?	76
„ *leptolepis*	Roche 202	+?	+?	76
Picea excelsa	Roche 202	—	—	76
Pinus lambertiana	i.b.a.	+		120
„ *maritima* *)	sol. of i.a.a.	+		35
„ *monticola*	i.b.a.	—		120
„ *silvestris*	Roche 202	—	+	76
Pseudotsuga douglasii	i.b.a.	—		120
MONOCOTYLEDONES				
Agrostis tenuis *)	talc powders containing n.a.a. or n.a.a. mixed with t.u.; Auxan, Hormodin, Rootone	—		43
Allium cepa	Germisan powder with n.a.a.		+?	5
„ „ *)	Merck and Rootone dust; sol. of n.a.a.K	—		13
„ „	sol. of i.a.a.		+	90, 91
„ „	sol. of i.a.a. + vit. C + t.u.		+	97
„ *libani* *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		7, 8
„ *porrum*	sol. of n.a.a.K + vit. C + t.u.		+?	5
„ „	sol. of i.a.a.		+	90, 91
„ „	sol. of i.a.a., i.a.a.K, n.a.a., n.a.a.K	—		115
Amaryllis belladonna *)	Rootone powder	+		69
Asparagus officinalis *)	Belvitan sol.	+		9
Avena sativa	sol. of i.a.a.		+	23
„ „	sol. of i.a.a.		+	25, 26
„ „ *)	sol. of e.i., i.a.a., i.m.m.a., i.p.a.	—	—	36, 37
„ „	sol. of i.a.a.	—	—	52
„ „	damage caused by disinfectants reduced by addition of i.a.a., n.a.a.	+	+	59
„ „ *)	sol. of i.a.a.		—	68
„ „	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—	—	71

[1]) List of abbreviations and commercial preparations on p. 2.

[2]) Results have been divided into effects upon *germination* and upon *development and yield.* A positive result, in the opinion of the author cited, is marked by a +; no effect, or a negative one, by a —, while +? indicates that the investigator himself does not attach much importance to the positive results. ? signifies lack of further indications.

*) Of this plant the scientific name was mentioned in the paper cited. The other plant names have been translated from the native names which includes the possibility that a wrong interpretation is given.

Species	Treatment	Results		References
		1. in germination	2. in development and yield	
Avena sativa	sol. of i.a.a.	+	+	74
„ „	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
„ „	talc powders and sol. containing i.a.a., i.b.a., n.a.a., n.a.d., ph.a.a.		—	106
„ „	Agrosan and talc powder with n.a.a.		—	112
„ „ *)	sol. of i.a.a.		+	113
„ „ var. *Fulghum* *)	sol. of i.a.a.		+	1
„ sp. *)	Merck and Rootone dust	—	—	13
Callicore Brunsvigia hybr. *)	Rootone powder	+		69
„ *rosea* = *Amaryllis belladonna* *)				
Cattleya warneri *)	i.b.a., n.a.a.	+	+	82
Festuca pratensis *)	Merck and Rootone dust	—	—	13
Gladiolus sp. *)	Rootone powder	—		69
Haemanthus katherinae *)	Rootone powder	+		69
Hordeum vulgare	powders with i.a.a. or n.a.a.	—	—	11
„ „	sol. of c.p.a., i.a.a., n.a.a., n.o.a.a., t.i.b.a.	—		15
„ „	sol. of i.a.a.		—	25
„ „	talc powders containing i.a.a., i.b.a., n.a.d.	—	—	31
„ „	mercurial disinfectant powder with i.a.a. or n.a.a.		+	56
„ „	talc powder with i.a.a.	—	+?	66
„ „	sol. of 2,4-D	+		67
„ „	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—	—	71
„ „	powders and sol. with i.a.a., n.a.a.	—	—	73
„ „	sol. of i.a.a.		+	96
„ „	Agrosan and talc powder with n.a.a.		—	112
Hordeum sp. *)	Mn, Cu and Fe salts of n.a.a.	—	?	86
Leucojum sp.	sol. of n.a.a., n.a.d., Roche 202	—		117
Lilium auratum *)	sol. of different growth substances	—		14
„ *regale* *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		7, 8
Lolium multiflorum	talc powders containing i.b.a., n.a.a. or n.a.a. + t.u.; Rootone and Hormodin A	—	+	29
„ *perenne* *)	Merck and Rootone dust	—	—	13
Moraea sp. *)	Rootone powder	—		69
Oryza sativa	sol. of i.a.a., n.a.a.	+	+	33
„ „	sol. of i.a.a.	—	—	52
„ „	sol. of 2,4-D	+		67
„ „ *)	sol. of i.a.a.	—	—	78
„ „	sol. of i.a.a.		+	84
„ „	sol. of i.a.a.	—		102
„ „ *)	sol. of i.a.a., i.b.a., n.a.a.		+	110
Panicum miliaceum	sol. of i.a.a.		—	23

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Panicum miliaceum	sol. of i.a.a.		—	25
„ „	sol. of i.a.a.	—		46
Poa pratensis *)	Merck and Rootone dust	—	—	13
„ „	talc powders containing i.b.a., n.a.a. or n.a.a. + t.u.; Rootone and Hormodin A	—	—	29
„ „ *)	talc powders containing n.a.a. or n.a.a. mixed with t.u.; Auxan, Hormodin, Rootone	—		43
Secale cereale	Germisan powder with n.a.a.		+?	5
„ „ *)	Merck and Rootone dust	—	—	13
„ „	sol. of i.a.a.		—	25
„ „	talc powders containing i.a.a., n.a.d.	—	—	31
„ „	sol. of i.a.a.		—	98
„ „ *)	sol. of n.a.a., n.a.d., Roche 202	+	—	117
Sorghum sp.	talc powder with 2,4-D		+	27
„ *sudanense*	sol. of i.a.a.		+	91
Sprekelia sp. *)	Rootone powder	+	—	69
Streptanthera sp. *)	Rootone powder	—		69
Triticum aestivum *)	talc powders containing i.a.a., i.b.a., n.a.a. Rootone. Combinations with Semesan	—	—	119
„ „ *)	sol. of n.a.a., n.a.d., Roche 202	+	—	117
„ *spelta*	sol. of n.a.a., n.a.d., Roche 202		—	117
„ *vulgare* (= *T. aestivum*)	sol. of i.a.a.K, i.b.a.K, n.a.a.K		+?	9
„ „ *)	sol. of i.a.a., ph.a.a. and mixtures	—	+	18
„ „	sol. of i.a.a.		—	23
„ „	sol. of i.a.a.		—	25
„ „	talc powder with 2,4-D		+	27
„ „	talc powders containing i.a.a., i.b.a., n.a.d.; Ceresan + growth substance		+	31
„ „ *)	sol. of Rootone		+	34
„ „	sol. of i.a.a.	—		46
„ „	sol. of i.a.a., n.a.a.	—		50
„ „	sol. of i.a.a.	+	+	74
„ „ *)	sol. of i.a.a.	—	—	78
„ „	Ceresan powder with i.a.a., n.a.a. and mixtures		+	80
„ „	sol. of i.a.a.	—		87
„ „	sol. of i.a.a.		+	90, 91, 92, 93, 95
„ „	sol. of i.a.a., n.a.a., n.a.a. + vit. B_1 and C	+	+	96
„ „	Agrosan, Granosan and talc-powder containing i.a.a., n.a.a.		—	112
„ „	fungicidal dust containing i.a.a., n.a.a.	+		116
„ „	sol. of n.a.a., n.a.d., Roche 202	+	—	117
„ „ *)	talc powders and sol. containing n.a.a. to reduce damage caused by hot water disinfection	—	—	**)

**) Unpublished results of experiments by the author in collaboration with Ir W. Kakebeeke.

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Triticum sp. *)	sol. of i.a.a., i.a.a.K, i.b.a., n.a.a., n.a.a.K; Merck and Rootone dust	—	—	13
„ „	mercurial disinfectant powder with i.a.a. or n.a.a.		+	56
„ „	damage caused by disinfection reduced by addition of i.a.a., n.a.a., ph.a.a.		+	58
„ „	damage caused by disinfectants reduced by addition of i.a.a., n.a.a.	+	+	59
„ „	formaldehyde containing i.a.a. or n.a.a.	+		60
„ „	formaldehyde containing n.a.a.	+	+	61
„ „	talc dust with i.a.a., n.a.a.K and combinations with KNO_3 and ethyl-mercuric bromide	—	—	62
„ „ *)	Mn, Cu and Fe salts of n.a.a.	+		86
„ „ *)	sol. of i.a.a.		+	113
Tritonia sp. *)	Rootone powder	—		69
Zea mays	Germisan powder with n.a.a.		+?	5
„ „	sol. of i.a.a.		+?	9
„ „ *)	sol. of i.a.a.		+	22
„ „	Rootone dust and mixture with Ceresan	—	+	30
„ „	sol. of i.a.a.	—		46
„ „	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—	—	71
„ „ *)	sol. of i.a.a.	—	—	78
„ „	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
„ „	talc powders with n.a.a.		—	106
DICOTYLEDONES				
Achillea ptarmica *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+		24, 109
Alyssum saxatile *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+		24, 109
Anemone pulsatilla rubra *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Anthemis tinctoria kelwayi *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Antirrhinum majus *)	Merck and Rootone dust; sol. of n.a.a.K	—	—	13
„ „	Rootone powder	+	+	69
Apium graveolens	sol. of n.a.a.K + vit. C + t.u.		+?	5
„ „	sol. of i.a.a.		+	91
Aquilegia "Crimson Star" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Arachis hypogaea *)	Merck and Rootone dust; sol. of n.a.a.K	—		14
„ „	lanolin with i.a.a.	+		100

Species	Treatment	Results		References
		1. in germination	2. in development and yield	
Arachis hypogaea	sol. of i.a.a.		+	101
Artemisia vulgaris *)	Belvitan sol.	+		9
Asclepias tuberosa *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+		24, 109
Atropa belladonna *)	Belvitan sol.	—	+	9
Aubrietia deltoidea "*Monarch Mixture*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Beta vulgaris *)	Merck dust; sol. of n.a.a.K	—	—	13
„ „	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
„ „	sol. of i.a.a., i.a.a.K, n.a.a., n.a.a.K	—		115
„ „ var. *altissima*	sol. of n.a.a.K		+	2
„ „ „ „	sol. of Euradin		+	3
„ „ „ „	sol. of n.a.a.K	?	?	4
„ „ „ „	Germisan powder with n.a.a.		+	5
„ „ „ „	sol. of i.a.a.		+	7, 8
„ „ „ „	sol. of i.a.a.K, i.b.a.K, n.a.a.K		+	9
„ „ „ „	sol. of n.a.a.K		+	10
„ „ „ „	sol. of n.a.a.K		—	20
„ „ „ „	sol. of i.a.a., n.a.a., combination with adenine, vit. B_1 and nicotinic acid		+	21
„ „ „ „	sol. of i.a.a. + adenine + vit. B_1 + nicotinic acid		+?	38
„ „ „ „	sol. of i.a.a., n.a.a., Euradin	—	—	39
„ „ „ „	sol. of n.a.a., Euradin, mixture of adenine + vit. B_1 + nicotinic acid + i.a.a.		+	40
„ „ „ „	different commercial preparations	—	—	41
„ „ „ „	sol. of n.a.a.		—	42
„ „ „ „	sol. of i.a.a., i.b.a., n.a.a., ph.a.a. and commercial preparations		—	44
„ „ „ „	sol. of n.a.a.	—	—	47
„ „ „ „	sol. of i.a.a., n.a.a.	—	+?	48
„ „ „ „	talc powders with i.a.a., n.a.a. and mixtures; sol. of i.a.a., n.a.a., i.a.a. + n.a.a., and combinations with t.u.; Euradin	—	—	49
„ „ „ „	sol. of i.a.a., n.a.a.		+?	51
„ „ „ „	sol. of n.a.a.		—	55
„ „ „ „	sol. of i.a.a., n.a.a.		—	63
„ „ „ „	sol. of Euradin		+	81
„ „ „ „	sol. of n.a.a.K		+	85
„ „ „ „	Hormodin powder; sol. of Hormodin A		—	89
„ „ „ „	sol. of i.a.a., n.a.a.	+	+	90
„ „ „ „	sol. of i.a.a.		+	91
„ „ „ „	talc powders and sol. with i.a.a., n.a.a.		+	97

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Beta vulgaris var. *altissima*	sol. of i.a.a., i.b.a., n.a.a., ph.a.a., Euradin, Belvitan, Roche 202		—	105
„ „ „ „	talc powder with n.a.a.; sol. of i.b.a., n.a.a.		—	106
„ „ „ „	talc powders and sol. containing i.a.a., i.b.a., n.a.a., n.a.d. Rootone		—	108
„ „ „ „	Agrosan and talc powder containing n.a.a.		—	112
„ „ „ „	sol. of i.a.a., n.a.a.		+	121
Boltonia asteroides *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Brassica campestris subsp. *chinensis* *)	sol. of i.a.a.	—	—	78
Brassica oleracea	sol. of n.a.a.K + vit. C + t.u.		+	5
„ „ *)	sol. of i.a.a.K	+		7
„ „ *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		8
„ „	Belvitan sol.	+		9
„ „	sol. of i.a.a.	—		46
„ „	sol. of i.a.a.		+	90, 91, 96, 97
„ „ *)	sol. of i.a.a., n.a.a.K		+	94
„ „ var. *acephala*	Belvitan sol.	+		9
„ „ „ *capitata*	sol. of i.a.a.		+	90, 96
„ „ „ „ *)	sol. of i.a.a., n.a.a.K		+	94
„ „ „ *capitata* f. *alba*	sol. of i.a.a.		+	91
Brassica oleracea var. *gongylodes*	sol. of n.a.a.K + vit. C + t.u.		+?	5
„ „ „ „	sol. of i.a.a.	—		46
„ „ „ „	sol. of i.a.a.		+	90, 91, 96
„ „ „ „	sol. of i.a.a., n.a.a.K		+	94
„ *rapa* *)	Merck dust; sol. of n.a.a.K	—	—	13
„ „	talc powder with n.a.a.; sol. of i.b.a., n.a.a.		—	106
Calendula officinalis	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
Cannabis sativa	sol. of i.a.a.		—	23
„ „	sol. of i.a.a.		+	27
Capsicum frutescens	sol. of i.a.a.	?	?	96
Carya illinoensis *)	sol. of i.b.a., n.a.a.	—		19
Chaenomeles japonica *)	sol. of n.a.a., n.a.d., Roche 202	—		117
Cheiranthus linifolius *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24
Chrysanthemum coccineum "*James Kelway*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+	+	109
Cichorium endivia *)	Belvitan sol.	+	+	9
„ „ *)	sol. of i.a.a.	—		46
„ *intybus*	Germisan powder with n.a.a.		+?	5
„ „ *)	Belvitan sol.	+	+	9
„ „	sol. of i.a.a.		+	91
Clematis tangutica *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109

Species	Treatment	Results		References
		1. in germination	2. in development and yield	
Coreopsis lanceolata "*Double Sunburst*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Cornus florida *)	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—		14
„ *stolonifera* *)	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—		14
Cotoneaster divaricata *)	talc powder with i.a.a. or n.a.d.	—		24, 109
„ *foveolata* *)	talc powder with i.a.a. or n.a.d.	—		24, 109
Cucumis sativus	Germisan powder with n.a.a.		+	5
„ „ *)	Belvitan sol.	+		9
„ „ *)	sol. of i.a.a.	—		70
„ „	sol. of i.a.a.		+	96, 97
„ „	sol. of i.a.a., n.a.a.		+	115
Cucurbita pepo *)	sol. of i.a.a.	—		104
„ „	talc powder with n.a.a.; sol. of i.b.a., n.a.a.		—	106
Cynara cardunculus *)	Belvitan sol.	+	+	9
Daphne mezereum *)	Belvitan sol.	+	+	9
Datura stramonium *)	sol. of i.a.a.K	—		7
„ „ *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		8
Daucus carota	sol. of n.a.a.K + vit. C + t.u.	?	?	4
„ „	Germisan powder with n.a.a.; sol. of n.a.a.K + vit. C + t.u.		+	5
„ „ *)	Belvitan sol.	+		9
„ „	sol. of i.a.a.	—		74
„ „	sol. of i.a.a.		+	90, 91
„ „	sol. of i.a.a., n.a.a.	—	+	96
„ „ *)	sol. of i.a.a.K + vit. B_1, vit. C, oestrone, caffeine and nicotinic acid		+	103
„ „	talc powder with n.a.a.; sol. of i.b.a., n.a.a.		—	106
„ „	sol. of i.a.a., i.a.a.K, n.a.a., n.a.a.K	—		115
„ „	sol. of n.a.a., n.a.d., Roche 202		+	117
„ „ var. *sativa* *)	Merck dust; sol. of n.a.a.K	—	—	13
Delphinium ajacis *)	sol. of i.a.a. or i.a.a.K	+		7, 8
„ „ *)	Merck and Rootone dust; sol. of n.a.a.K	—	—	13
„ "*Martin's Stock*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+		24, 109
Dianthus alpinus allwoodii *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—	+	24, 109
Digitalis lanata *)	sol. of n.a.a.	±		115
„ *purpurea gloxiniflora* "*Rose*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Diospyros virginiana *)	talc powder with i.a.a. or n.a.d.	—		24, 109
Erigeron coulteri *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Erysimum linifolium *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		109
Fagopyrum esculentum	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
„ „	talc powders with n.a.a.; sol. of i.b.a., n.a.a.		—	106
Geum chiloense "*Orange Queen*"*)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Glycine = *Soja*				
Gossypium sp.	dust containing i.b.a., n.a.a.K and combinations with Spergon and Ceresan	—	—	75
„ „	sol. of i.a.a.		+	101
Helianthemum polifolium *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		7, 8
„ *roseum* *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		7, 8
„ *vulgare* *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		7, 8
Helianthus annuus *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		8
„ „ *)	sol. of i.a.a.	—		104
Heliopsis pitcheriana *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Heuchera lithophila *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		109
Ilex opaca *)	talc powder with i.a.a. or n.a.d.	—		24, 109
Impatiens sp.	sol. of n.a.a., n.a.d., Roche 202	—		117
Incarvillea delavayi *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Inula helenium *)	Belvitan sol.	+	+	9
Lactuca sativa	Germisan powder with n.a.a.; sol. of n.a.a.K + vit. C + t.u.		-?	5
„ „ *)	Merck and Rootone dust; sol. of i.a.a.K, n.a.a.K	—		13
„ „	sol. of i.a.a.		+	90, 91, 96, 97
Lavendula spica *)	Belvitan sol.	+		9
Lens sp. *)	Mn, Cu and Fe salts of n.a.a.	—	?	86
Lepidium sativum *)	sol. of e.i., i.a.a., i.m.m.a., i.p.a.	—		36, 37
„ „	sol. of i.a.a., i.p.a.		+	79
Liatris pycnostachya *)	talc powders containing i.a.a., or n.a.d. mixed with t.u.	—		24, 109
Linum usitatissimum	sol. of i.a.a.		—	23
„ „	sol. of i.a.a.		+	90, 91
„ „	sol. of i.a.a.	—		102
Lobelia cardinalis *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Lupinus luteus *)	sol. of n.a.a., Roche 202	+	+	117
„ *polyphyllus* *)	sol. of n.a.a.		+	117
„ „ "*Blue King*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109

Species	Treatment	Results		References
		1. in germination	2. in development and yield	
Lupinus polyphyllus "*Rose Queen*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	—		24, 109
Lychnis arkwrightii *)	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	—		24, 109
Lycopersicon = *Solanum*				
Lythrum salicaria roseum superbum *)	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	—		109
Maclura pomifera *)	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—		14
Matricaria inodora "*Dwarf Golden Ball*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	—		24, 109
Matthiola incana *)	Merck and Rootone dust; sol. of n.a.a.K	—	—	13
Medicago sativa	sol. of i.a.a.K		+	9
Melissa officinalis *)	Belvitan sol.	+	+	9
Mespilus germanica *) . . .	sol. of n.a.a., n.a.d., Roche 202	—		117
Nicotiana tabacum	Germisan powder with n.a.a.; sol. of n.a.a.K + vit. C + t.u.		+?	6
„ „	i.a.a., 2,4-D		—	65
Ocimum basilicum *)	Belvitan sol.	+	+	9
Oenothera fruticosa youngii *) .	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	—		24, 109
Orlaya grandiflora *)	sol. of i.a.a.	+		8
Paeonia suffruticosa *)	sol. of i.a.a.K, n.a.a.K . . .	—		13, 14
Papaver nudicaule "*Stanford's Giant*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	+		24, 109
„ *somniferum*	Germisan powder with n.a.a.		+	5
„ „ *)	sol. of i.a.a.		+	91
Pentstemon gloxinoides "*Sensation*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	+		24, 109
Perilla ocymoides	sol. of i.a.a.		—	23
„ „	sol. of i.a.a.		+	27
Petroselinum sativum *) . . .	sol. of i.a.a.K	+?		7
„ „ *) . . .	Belvitan sol.	+	+	9
„ „	sol. of i.a.a.		+	90, 91
„ „	sol. of i.a.a., i.a.a.K, n.a.a., n.a.a.K	—		115
Phacelia tanacetifolia *) . . .	talc dust and sol. with i.a.a.		+	96
Phaseolus angularis *)	sol. of i.a.a.		—	118
„ *lunatus*	Rootone dust	—		30
„ *mungo* var. *radiatus* *)	sol. of i.a.a.	+	—	78
„ *vulgaris*	sol. of i.a.a.	+		74
„ „	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d. . .		—	83
„ „ *)	sol. of i.a.a.	—		104
„ sp.	Rootone dust; mixture with Ceresan	—	—	30
Phaseolus sp.	dry seed dressings (mercurial and cuprous oxide) containing n.a.a., mixed naphthylidene acetic acids and i.b.a. . . .	—	—	32
Physostegia virginiana grandiflora *)	talc powders containing i.a.a. or n.a.d. mixed with t.u. . .	+		109

Species	Treatment	Results		References
		1. in germination	2. in development and yield	
Physostegia virginiana rosea *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24
Phytolacca decandra *)	sol. of i.a.a., i.b.a., n.a.a.	—	—	13
Pisum sativum *)	Merck and Rootone dust; sol. of n.a.a.K	—		13
„ „ *)	sol. of i.a.a., ph.a.a.	—	+	16
„ „ *)	sol. of i.a.a. and ph.a.a. in combination with manganese sulphate and uranyl nitrate	—	+	17
„ „	sol. of i.a.a.		—	23
„ „	sol. of 2,4-DNH_4	?	?	28
„ „	Rootone dust	—	+	30
„ „	dry seed dressings (mercurial and cuprous oxide) containing n.a.a., mixed naphthylidene acetic acids and i.b.a	+	+	32
„ „	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
„ „	talc dust containing i.a.a.		+	97
„ „ *)	sol. of i.a.a.	—		104
„ sp. *)	Mn, Cu and Fe salts of n.a.a.	—	?	86
Platycodon grandiflorum mariesii *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Polemonium richardsonii *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+		24, 109
Potentilla nepalensis *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+		24, 109
Prunus persica	sol. of i.b.a.	—		99
Pyrethrum = *Chrysanthemum*				
Pyrus malus var. *niedwetzkyana* *)	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—		14
„ sp. *)	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—		14
Raphanus sativus *)	sol. of i.a.a.K	+	+	7
„ „ *)	Merck and Rootone powder; sol. of n.a.a.K	—	+	13
„ „	sol. of i.a.a., i.b.a., n.a.a.	—	—	53
„ „	sol of i.a.a.	+		74
„ „	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
„ „	sol. of i.a.a.K, n.a.a.K		+	88
„ „	sol. of n.a.a.K and combinations with vit. C, t.u. and i.b.a.K		+	94, 96
„ „	talc powders and sol. containing i.a.a., i.b.a., n.a.a., n.a.d., n.o.a.a., ph.a.a.		—	106
„ „	sol. of i.a.a., i.a.a.K, n.a.a., n.a.a.K	—		115
Rhododendron obtusum kaempferi *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		109
Ricinus communis *)	sol. of i.a.a.	—		104
Rudbeckia hirta hybr. "*Autumn Forest*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109

Species	Treatment	Results 1. in germination	Results 2. in development and yield	References
Satureja hortensis *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		7, 8
Saxifraga cordifolia *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Silene saxifraga *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Sinapis alba (=*Brassica alba*)	sol. of i.a.a.		+	23
„ „ *)	sol. of c.i., i.a.a., i.m.m.a., i.p.a.	—	—	36, 37
„ „ *)	sol. of i.a.a., i.b.a., n.a.a.		+	110
„ „ *)	sol. of i.a.a.Na, n.a.a.Na		—	111
Soja max (=*Glycine max.* = „ *soja*) *)	sol. of i.a.a., i.b.a., n.a.a., and mixtures	+		7, 8
„ „ *)	Merck and Rootone dust	—		13
„ „	sol. of i.a.a.	—		46
„ „	talc powder with i.a.a.; sol. of i.a.a.	—	+	56
„ „	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	—	—	71
„ „	talc powders with i.a.a., i.a.d., i.b.a., i.p.a., n.a.a., n.a.d.		—	83
„ „	talc powder with n.a.a.; sol. of i.b.a., n.a.a.		—	106
„ „ *)	sol. of i.a.a.		—	118
„ „ *)	talc powders containing i.a.a., i.b.a., n.a.a.; Rootone, combinations with Semesan	—	—	119
Solanum melongena *)	Belvitan sol.	+	+	9
„ „ var. *esculentum* *)	Merck and Rootone powder; sol. of n.a.a.K	—		13
„ *lycopersicum*	sol. of n.a.a.K + vit. C + t.u.	?	?	4
„ „ *)	Merck and Rootone dust; sol. of i.a.a., i.a.a.K, i.b.a., n.a.a., n.a.a.K	—	—	13
„ „	sol. of i.a.a., i.b.a., n.a.a.	—	+	53
„ „	sol. of i.a.a.	—	—	64
„ „	sol. of i.a.a.	+		74
„ „	sol. of i.a.a.		+	91
„ „	talc powders with i.a.a., i.b.a., n.a.a., ph.a.a.	+	+	107
„ „ *)	sol. of i.a.a., i.b.a., n.a.a.		+	110
„ „ *)	sol. of i.a.a.		+	113
„ „	sol. of i.a.a., n.a.a.		+	115
„ „ *)	talc powder with n.a.a.; sol. of i.a.a.	—	—	***)
„ *tuberosum*	sol. of i.a.a., i.b.a., n.a.a.		—	53
Spinacia oleracea	Germisan powder with n.a.a.		+	5
„ „ *)	Belvitan sol.	+		9
„ „	sol. of i.a.a.		+	27
„ „	sol. of i.a.a., i.a.a.K, n.a.a., n.a.a.K	—		115

***) Unpublished results of experiments by the author.

Species	Treatment	Results		References
		1. in germination	2. in development and yield	
Tagetes erecta *)	Merck and Rootone powder; sol. of n.a.a.K	—		13
„ *patula* *)	Merck and Rootone powder; sol. of n.a.a.K	—		13
Thalictrum aquilegifolium *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		109
Thymus vulgaris *)	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		7, 8
„ „ *)	Belvitan sol.	+	+	9
Trifolium hybridum	sol. of i.a.a., i.b.a., n.a.a. and mixtures	+		8
Trollius europaeus *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		109
Tropaeolum majus *)	Merck and Rootone dust; sol. of n.a.a.K	—	—	13
Ulmus americana *)	sol. of i.a.a., i.b.a., n.a.a., ph.a.a.	+		14
Valeriana officinalis	sol. of i.a.a.	—		46
Valerianella olitoria *)	Belvitan sol.	+	+	9
Veronica longifolia hendersonii *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24, 109
Viburnum lantana *)	talc powder with i.a.a. or n.a.d.	—		24, 109
„ sp. *)	sol. of different growth substances	—		14
Vicia faba *)	sol. of i.a.a.		—	68
„ „ *)	Mn, Cu and Fe salts of n.a.a.	—	?	86
„ *sativa*	sol. of i.a.a.		—	23
Viola arkwright rubra *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		24
„ *cornuta chantreyland* *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	+		24, 109
„ „ "*Rose Queen*" *)	talc powders containing i.a.a. or n.a.d. mixed with t.u.	—		109
Zinnia sp. *)	Merck and Rootone dust; sol. of n.a.a.K	—	—	13

Chapter II

GENERAL METHODS

§ 1. Material

The common pea was used, Nr 756, "Groene Lente" from the firm of Messrs. Turkenburg, of Bodegraven, Holland. Usually seeds were chosen from the latest crop available.

§ 2. Sterilization and pre-soaking

As mere traces of some ergons may influence the development of the embryo, it is obvious that the present investigation can be made *in vitro* only, under strictly aseptic conditions. Many micro-organisms produce substances which have been proved growth factors for higher plants, thus their presence would confuse the results.

The peas were sterilized externally by immersion in alcohol (96 %) for 10 minutes, in $HgCl_2$ (0.1 %) for 15 minutes and by washing four times with sterile aq. dest. Then the seeds were pre-soaked in sterile water or in sterile solutions of the compounds under investigation (growth substances, inhibitors, etc., of various concentrations) by keeping them in sterilized Petri dishes at about 24° C for some 18 hours (i.e. overnight). After carefully washing the seeds under sterile conditions the embryo*) was separated from the cotyledons. The instruments were sterilized by dipping in alcohol (96 %) and subsequent flaming before each operation. In a number of series, parts of the cotyledons were left attached to it. Only those seeds were used, the coat of which was not ruptured after soaking.

Attempts at isolation of the embryo from seeds, not soaked beforehand, were unsuccessful. The embryo in dry seed being very brittle, a slight injury during the manipulations is hardly preventable. Uninjured embryos possibly might be isolated from the dry, hard seeds by means of a special, though time-devouring technique (243) which, however, never would provide the material required for sufficiently large experiments.

All the operations were carried out in a transfer room previously sterilized by U.V. lamps.

§ 3. Media and culture

Culture, under sterile conditions, took place in the dark at 24° C. Culture tubes were used, containing 10 ml of a standard nutrient medium according to Bonner and Addicott (232). It is composed as follows:

$Ca(NO_3)_2.4H_2O$	236 mg
$MgSO_4.7H_2O$	36 „
KNO_3	81 „
KCl	65 „
KH_2PO_4	20 „
$Fe_2(SO_4)_3$	2 „
sucrose	40 g
double-distilled water, up to	1000 ml
agar	±4 g

$Ca(NO_3)_2.4H_2O$ was prepared from $CaCO_3$ (A.R.) and nitric acid (halogen-free); crystallized from water.

KH_2PO_4 was prepared from K_2CO_3 (pure grade, from tartar) and H_3PO_4 (pure grade); recrystallized till meeting buffer grade specifications.

The sucrose employed was commercial sugar, whereas commercial agar, before use, was thoroughly leached with distilled water for a week.

The synthetic growth substances, di-n.amyl-acetic acid, rac. (trans)-tetrahydrothiophene-α, α'-dicarboxylic acid and the cis isomer were prepared in the Research Laboratory of the N.V. Amsterdamsche

*) "embryo" in the text stands for "embryo devoid of cotyledons".

Chininefabriek, Amsterdam. All the other chemicals were of the purest grade available.

Sterilization, as a rule, was effected by autoclaving at 110° C for 15 minutes. If thermolabile substances had to be added to the nutrient medium, 5 ml portions of a solution containing twice the amount of sugar, salts and agar were sterilized in the tubes. Before cooling, the substance under investigation was added, viz. 5 ml of a solution sterilized by filtration through a sintered glass funnel (17 G 5, SCHOTT, Jena).

§ 4. ESTIMATION OF RESULTS

Biological materials always show variability to a large extent. In order to obtain results reliable statistically, groups were made up of 20 specimens at the least.

After culture for ten days or three weeks, dependent on the kind of the experiment, abnormal plants were discarded and results were recorded photographically. Lengths of shoot, hypocotyl and root were measured and, the numeration of lateral roots being performed, fresh and dry weight determinations were carried out.

The standard error was used, if possible, as an estimate of the dispersion of the means and it was calculated according to the equation

$$E = \pm \sqrt{\frac{\Sigma d^2}{n(n-1)}}.$$

CHAPTER III

PRELIMINARY EXPERIMENTS

§ 1. IMPORTANCE OF THE SIZE OF SEED AND EMBRYO

Any given lot of peas will consist of seeds of rather varying size. It is imaginable that the seeds of such an assortment may develop unequally, owing to a different content of ergons and of reserve substances, and on account of disparate size of the embryos. In the literature this theme is mentioned several times (F, 200–212), though any possible bearing upon the *in vitro* culture of embryos or of roots has been ignored.

Hence, in the present investigation the material was divided into five lots by means of thin, wooden sieves, fit with holes 5.5, 6.0, 6.5 and 7.0 mm wide. So, lots resulted of the following diameters: under 5.5, 5.5–6.0, 6.0–6.5, 6.5–7.0 and over 7.0 mm.

After a night's pre-soaking it is distinctly visible that the larger peas have swollen most and have been the first to rupture the seed-coat and to show the root-tip, whereas the smaller peas have swollen least and have not ruptured.

During pre-soaking of peas of a size, differences prove to appear since not all of them will swell to the same degree. Prior to the excision of embryos, therefore, the material must be sized once more. As already stated, seeds were chosen the coat of which was not yet ruptured.

TABLE III

Ratio of embryo to mass of reserve substances after pre-soaking. Average of 100 specimens. Weights in mg.

Diameter of the seed	Weight of embryo		Weight of cotyledon				Ratio: embryo / reserve substances	
			fresh		dry			
	fresh	dry	left *)	right *)	left *)	right *)	fresh	dry
< 5 mm	4.8	1.2	97.8	99.5	39.4	40.0	1 : 41	1 : 68
6.0-6.5 mm	6.1	1.7	157.0	153.5	69.5	68.5	1 : 51	1 : 82
> 7 mm	7.7	2.2	248.7	248.0	114.3	114.6	1 : 65	1 : 102

*) see fig. 1.

It appears that, in peas of different diameter, both the size of the embryo and the ratio embryo/reserve substances widely diverge (see table III) and that these large differences lead to unequal development (table IV).

§ 2. Effect of age and storage conditions

It is obvious to assume that the age of the seeds may be of consequence to the process of germination and to further development. In 1952 an experiment was made with peas of one size, harvested in 1945, 1947, 1949 and 1951, respectively. Only minor differences in development were seen.

Nevertheless, errors if any from this source were eliminated by always using seeds from the latest crop available. Cool and dry storage was maintained as much as possible.

§ 3. Influence of pre-soaking

A short period of soaking was deemed indispensable for the excision of embryos in undamaged condition. The method, for all that, is liable to objections. For, it is not only thinkable, but also probable that already during pre-soaking certain components from the cotyledons will be absorbed by the embryo (see 238), in which case results are not unequivocal.

So, it seemed worth while studying the factors involved in pre-soaking as well as their importance. Soaking was always performed in the dark.

a. Effect of the temperature

If, during pre-soaking, any substances are transferred from the cotyledons to the embryo, one might try to check this process, e.g. by lowering the temperature. On the other hand, there is some danger of certain enzymatic processes being favoured by low temperature (vernalization!), so that subsequent development would be influenced again.

Temperatures of 4°, 25°, 35° and 45° C were studied and so were various soaking-periods. The numerical data show that further development of the embryo is either not affected by the temperature

TABLE IV

Influence of the size of the seed on the ultimate development of the dissected embryo. Results after three weeks. Average of 35 specimens.

Diameter of the seed	Length of the embryo	Average weight of the two cotyledons after pre-soaking period		Sprout			Root + hypocotyl			Average number of lateral roots
		fresh	dry	length	fresh weight	dry weight	length	fresh weight	dry weight	
mm	mm	mg	mg	mm	mg	mg	mm	mg	mg	
< 5.5	4.0	215	87	61 ± 1.39	45	3	54 ± 0.98	26	3	8
5.5 - 6.0	4.5	257	106	66 ± 1.33	49	3	59 ± 1.34	29	3	9
6.0 - 6.5	5.0	318	135	73 ± 1.41	54	3	64 ± 1.69	33	4	9
6.5 - 7.0	5.0	375	138	75 ± 1.62	55	3	64 ± 1.04	32	4	10
> 7.0	5.5	478	209	78 ± 1.79	60	4	67 ± 1.34	36	4	10

during pre-soaking, or to a small degree only. Both the former and the latter temperature caused an increase of abnormal individuals. A lengthy treatment at 25° C was also fatal.

b. Duration of pre-soaking

For the above reasons pre-soaking must be stopped in good time. BONNER and BONNER (see 238) mention a period of six hours at most. At 25° C, it will require at least a five hours' soaking before enough seeds have swollen satisfactorily. The process takes even more time at 4° C; besides, the peas not only remain wrinkled a good long while, but also become tough and keep retaining a hard centre. As a rule, the embryo cannot then be properly excised.

In experiments, carried out at the same temperature, the duration of pre-soaking scarcely affected the development of the young plant. So, if any substances are transferred from the cotyledons to the embryo, it will take place already in the first stage of swelling. In *rye*, according to DE ROPP, the transport of growth-stimulating substances would take place within the first two hours (238).

c. Water-supply

When soaked in water, the seeds exude various substances. Peas not only give off inhibitory compounds: a red pigment was isolated from "pea-diffusate" by VELDSTRA.*)

It is quite possible, therefore, that the substances exuded will influence the embryo inside the seed if their concentration becomes too high or if pre-soaking is protracted. In Nature, after all, such substances are adsorbed by the soil, or decomposed by the action of micro-organisms.

Peas of one size, in lots of fifty, were pre-soaked in 10, 20 or 40 ml of water, under sterile conditions at 24° C. After this it appeared that in the first dish the peas had absorbed the whole of the water; in the next, enough water was left, whereas in the third dish the greater part of the peas had remained submerged, which involves some danger of "soaking injury" (190–199).

On the whole, the development of the embryos showed no large differences. So, if not too many peas are put together in one dish and if so much water is added that the peas are submerged only half, no substances given off by the seeds will influence the future development of the embryo.

§ 4. POSITION OF THE EMBRYO ON THE NUTRIENT MEDIUM

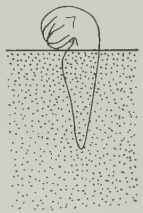
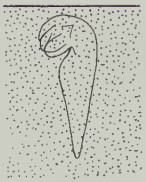
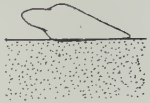
If an excised embryo is transferred into a culture-tube (containing a nutrient medium) by simply dropping, its position will wholly depend on chance. It is conceivable that the initial position may influence future development; therefore, it was tried to demonstrate this by a number of tests.

Starting from peas of 6.0–6.5 mm diameter the embryos were placed upon and into the nutrient medium in five, diverse positions: upright, its plumule rising above the medium; upright, completely immersed;

*) Unpublished.

TABLE V

Influence of the position of the embryo on the nutrient medium (at the beginning of the culture) upon ultimate development. Standard medium with 4% sucrose and 0.4% agar. Results after 21 days. Average of ± 30 specimens.

Position of the embryo at the beginning of the culture					
Sprout — average length . . . . . (mm)	78 ± 1.6	76 ± 1.6	70 ± 2.0	69 ± 1.5	64 ± 2.3
Sprout — „ fresh weight. . . . . (mg)	57	53	50	51	48
Sprout — „ dry weight . . . . . (mg)	3	3	3	3	3
Root plus hypocotyl — average length . . . . . (mm)	63 ± 1.4	62 ± 1.7	59 ± 1.4	62 ± 1.3	56 ± 0.9
Root plus hypocotyl — „ fresh weight. . . . . (mg)	34	35	26	27	26
Root plus hypocotyl — „ dry weight . . . . . (mg)	3	3	3	3	3
Average number of lateral roots	10.6	9.9	10.0	10.8	9.8
Percentage of plants with the root growing above the medium	0	0	10	38	20

lying on its back (i.e. ,the side facing the seed-coat in the whole pea, see fig. 1); with its back aloft, and, finally, lying on its side.

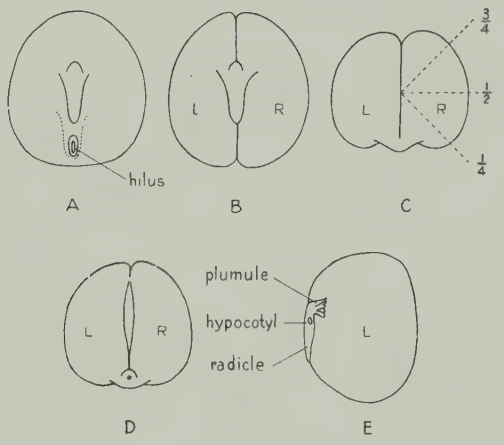


Fig. 1. Seed of pea. A, as seen from attached edge (back of the embryo). B, same as A, seed-coat removed. L = left cotyledon; R = right cotyledon. C, as seen from above, seed-coat removed. D, cross-section through plumule, seed-coat removed. E, seed-coat and right cotyledon removed.

From the results, summarized in table V, it may be concluded that development is influenced indeed by the position of the embryo. Especially in quantitative experiments, therefore, attention should be paid to this point.

§ 5. Effects of injury

Various workers have stressed the point that an embryo is very sensitive to certain manipulations. In order to check these statements, embryos, freshly excised, were either pricked with a needle or grasped with fairly hot tweezers before placing them into the culture-tubes. In comparing their development with that of embryos handled with utmost care, scarcely any trace of injurious after-effects was found in the former.

If, then, the embryos are isolated cautiously and if the instruments are allowed to cool after flaming no ill effects are to be feared.

§ 6. Influence of the medium; concentration of the agar

No doubt the composition of the medium will affect development. In this investigation the medium according to Bonner and Addicott (232) has been used, though, in another connection, it could be shown that its composition is certainly not optimal for the culture of pea-embryos. Besides, it is to be expected that the demands of the plant will vary during development.

Attempts at making embryos develop on a liquid medium have failed of effect. Finally, an agar medium was preferred for culture and its optimal concentration was ascertained.

As appears from fig. 2, the standard medium containing 4 % sucrose yields the best results both in sprout and in root development if about 0.3 % agar is present. A concentration of 0.2 % is generally too low; "drowning" of the embryo gives rise to abnormal development.

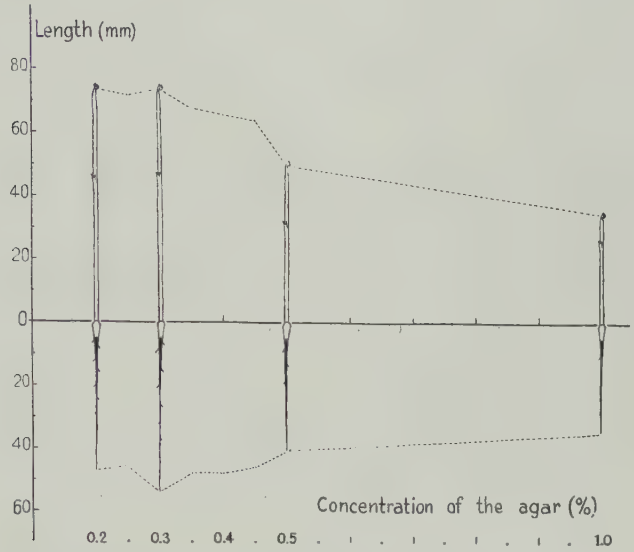


Fig. 2. Influence of different agar concentrations in the standard nutrient medium. Results after three weeks' culture in the dark at about 24° C.

§ 7. Aeration

It is obvious that during *in vitro* culture the gas-volume may play a part. Even a small variation of the diameter of the tubes leads to different levels of the medium, thus the space available to the aerial parts of the plant is different too. Trouble will be caused not only by mechanical influences (growth against the cotton plug and, therefore, immersion of the basal parts of the plant), but also by mutual variations of the composition of the gas-milieu.

In order to eliminate possible errors in advance, only tubes of one size were used (150 mm in length and 17–18 mm wide).

Another point requiring attention is the firmness of the cotton plug. It may, after all, affect the interchange with the air. Comparisons were made between plugs of the usual, medium texture and both very loose and very tight plugs. The results obtained with the various kinds were very much alike, so, from that time normal plugs were used, made up always by the same person.

Summary, and survey of methods

1. Peas, usually seeds of the latest crop available, were assorted by sifting shortly before use (cf. note on p. 61).

2. As a rule, peas of one size were employed. They were externally sterilized by immersion both in 96 % alcohol for 10 minutes and in 0.1 % $HgCl_2$ for 15 minutes, and wahsed four times with sterilized, distilled water.
3. To facilitate excision of the embryo, the peas were pre-soaked overnight in water or in certain solutions, at about 24° C, in the dark.
4. Seeds, of which the seed-coats had remained intact, were calibrated again before the excision of the embryos. After removal of the seed-coat, the cotyledon-stalks were cut close by the embryo, in order to obtain similar objects.
5. These embryos, after being placed upright into culture tubes, were adjusted right in the centre of the nutrient medium by means of a transfer loop, the plumule rising just above the surface.
6. A medium containing 0.3–0.4 per cent agar ensured optimal development, unless substances were present affecting the gelation properties.
7. In the proceedings mentioned under 4 and 5, the influence of the time-factor was limited as much as possible. If, for instance, a number of treatments was tested on 24 objects each, the successive manipulations were finished with 8 objects only of each group; this sequence was repeated until all of the material had been handled.
8. Culture took place in the dark, at about 24° C.
9. After ten days or three weeks, dependent on the kind of experiment, the results were recorded.

Chapter IV

EARLY DEVELOPMENT AND CORRELATION PHENOMENA; INFLUENCE OF VARIOUS SUBSTANCES

§ 1. Influence of the cotyledons

The cotyledon, as a depot of reserve substances and ergons, certainly will have a large influence upon normal development. If seeds are treated with growth substances, it may be imagined that their action on the embryo will not only be direct, but also indirect, via the cotyledons. For, the latter strongly swell when soaked in water or in growth substance solutions. Considering the volume of the cotyledons, being often much larger than that of the embryo (see table III, p. 24) it is quite possible that the after-effect of a growth substance treatment becomes very pronounced, if exerted via the cotyledons.

This may also account for the contradictory results obtained in testing several kinds of seeds. Blociszewski (231) already showed that in various seeds large differences do exist in the proportion size of embryo/volume of reserve substances:

Zea mais	1 : 4.73
Trifolium	1 : 5.66
Raphanus	1 : 5.76
Avena	1 : 15.75
Secale	1 : 17.25
Pisum	1 : 32.40
Lupinus	1 : 49.88

As to the pea, this statement was put to the test with seeds of various size (see p. 24).

The above discrepancies, however, need not only arise from a different proportion of embryo-volume to cotyledon-volume, perhaps *the nature of the reserve-substances in the cotyledons too may be determinative for the action of the growth substance.*

Prior to the treatment of this problem, an analysis was made of the correlation-phenomena appearing in early development and of their eventual influencing by constituents of the nutrient medium.

a. The early stages of development in the presence of various amounts of cotyledon-tissue

Information about the role of the cotyledons may be obtained, e.g., by determining the growth of both sprout and root in embryos, connected with different amounts of reserve substance tissue.

Partial amputation of the cotyledons of this object proved feasible, without functional disturbances appearing; that is to say, barring possible effects of reducing the amount of reserve substance tissue. For, the cotyledon of the pea, on microscopical examination, proves fairly homogeneous; inside the epidermis parenchyma is found, filled up with starch. Close by the attachment with the embryo some vascular tissue is present. In amputating, this part was always left intact, by cutting the cotyledon parallel to the embryo (see fig. 1, C).

For this purpose an experiment was made with peas, 6.0–6.5 mm in diameter, which, after sterilizing, were pre-soaked in distilled water. Five groups were arranged, consisting of: embryos without cotyledons, embryos with part of a cotyledon (a quarter, or a half) and embryos with either one or two complete ones. Whether a single cotyledon or part of it was left attached to the embryo, it was always on the right side (see fig. 1, C).

Cultivation was carried out in the dark, at about 24° C. The lengths of both the sprout and the root (including the hypocotyl) were measured regularly with a celluloid rule, while from time to time some objects were set apart for fresh and dry weight determinations.

This led to the following observations:

1. Development of plain embryos proceeds regularly. The more cotyledon-tissue is left, the larger the divergence between objects, otherwise treated similarly. Despite of the uniformity of the peas, their cotyledons are often mutually different. A different content of nutrients and growth factors probably will be the cause of variations in development.
2. Development starts with vigorous root-growth; not until the root has attained some length does sprout-growth begin.

3. Growth, of both the root and the sprout, is the more vigorous, the more cotyledon-tissue is left to the embryo. The mere presence of a small piece takes an enormous effect.

Therefore, in studying the influence upon isolated embryos, of substances added to the nutrient medium, one must cut the cotyledon-stalks close to the embryo, lest errors should appear in quantitative tests.

4. If cotyledon-tissue is present and the sprout grows against the cotton plug, so that both the hypocotyl and the basal part of the sprout are pressed down into the nutrient medium, the agar at once starts deliquescing, until a clear solution results. A preliminary communication (239) treats of the subject.

5. The more cotyledon-tissue is present, the less the boundary line between the hypocotyl and the root is distinguishable. The young plant, grown from an embryo without reserve food, shows a dark root, clearly discernible from the hypocotyl.

6. An embryo without cotyledons produces hardly any lateral roots (at best some tiny points, after three weeks), whereas the object, remained intact, possesses a large number of well-developed, lateral roots. Here too the amount of cotyledon-tissue is related to the average number of lateral roots. Most of the latter are usually located on the side where the cotyledon was left, though it is often difficult to judge of, owing to torsion of the main root.

Several of the correlation-phenomena outlined have been observed in seedlings by other authors, like DOSTÁL (213), KOŘÍNEK (215), MATON (216) and PLCH (217). It seemed desirable, however, to analyse these relations under the circumstances of the present experiments, in view of making comparisons when, afterwards, growth substances would come into play.

b. Influence of the cotyledons after amputations at different stages of development

The above results were obtained after the embryos, attached with cotyledon-tissue, had been cultivated for about eight days. In view of the subsequent tests with growth substances, it was deemed essential to determine the lapse of time, during which the cotyledon-tissue has to remain connected with the embryo, in order that, after amputation, a distinct growth-stimulating influence may be exercised.

Beside a group of twenty plain embryos, one hundred objects with both of the cotyledons were put into tubes and cultivated as usual. Every twenty-four hours 20 objects were deprived of their cotyledons, one group of 20 specimens being left intact for the sake of comparison. After nine days the results were recorded (table VI).

Just as in normal development, the growth of the root was stimulated before that of the sprout became vigorous. Evidently, the early growth processes were sufficiently stimulated if the connection of the cotyledons with the embryo was maintained for 24 hours.

The promotion of sprout development by the cotyledons went on as long as these organs were present (in this experiment for nine days).

TABLE VI

After-effect of cotyledons, amputated at various stages. Average of about 20 specimens. Results after nine days' cultivation on standard medium.

		Cotyledons amputated					
		at once after pre-soaking	after 24 hours	after 2 × 24 hours	after 3 × 24 hours	after 4 × 24 hours	not at all
Sprout	average length (mm)	33 ± 1.16	37 ± 1.83	55 ± 3.10	73 ± 2.62	95 ± 4.32	149 ± 5.33
	„ fresh weight (mg)	42	51	99	167	245	462
	„ dry weight (mg)	2	3	5	9	13	30
Root with hypocotyl	average length (mm)	37 ± 1.33	50 ± 2.47	68 ± 2.52	96 ± 1.81	97 ± 2.93	90 ± 3.33
	„ fresh weight (mg)	25	39	57	78	99	98
	„ dry weight (mg)	3	4	5	7	10	12
Average number of lateral roots		0.4	1.1	2.3	7.0	16.5	15.1

Root growth, however, came to a temporary stand-still after 3 × 24 hours already.

Furthermore, the formation and the growth of lateral roots were clearly influenced by the cotyledons. The average number of these roots was doubled every 24 hours, until the maximum (about 16) was reached after four days. The growth-promoting action of the cotyledons was dependent on a normal connection with the embryo: if amputated cotyledons or parts of them were placed into the medium, around the embryo, scarcely any influence upon root growth was observed.

c. Regeneration-phenomena after amputation of the plumule, in the presence of various amounts of cotyledon-tissue

An interesting correlation between the development of the sprout and that of the lateral roots reveals itself in the regeneration of the sprout, after the plumule has been amputated simultaneously with part of the cotyledon-tissue, viz. on one side of the embryo.

The matter was investigated further. Peas, 6.0–6.5 mm in diameter, were pre-soaked in water. Immediately after this, the left cotyledon was cut off in whole, whereas the right one was either curtailed (to one quarter, or one half, resp.) or kept intact. Beside these groups, with normal embryos, similar ones were arranged from which the plumule was removed as well.

After four days' culture in the dark, lateral roots began to appear, while after six days part of the objects, deprived of their sprouts, started developing a new one from the axillary bud of the failing cotyledon.

When the test (table VII) was finished after eight days, the largest number of lateral roots was found with the objects which had developed a new sprout, especially if parts of a cotyledon were present.

As to the development of lateral roots, the objects with intact sprouts differed the more from the amputated ones, the less cotyledon-tissue was present. No explanation can be offered for the present.

So, there is a distinct correlation between the growth of the regenerated sprout and the development of lateral roots.

A better understanding of the mutual influencing of sprout and root will not be gained until these organs have been cultivated, both separately and together, on the same nutrient medium, by the technique of organ-culture (240). STEPHENSON (218) showed for *lettuce* seedlings that, in a standard solution lacking certain growth-factors, root growth was promoted indeed if the sprout was present in the medium. The administration of nicotinic acid took a similar effect. On the other hand, the main root exerted a favourable influence on the formation of adventitious roots to the sprout. This stimulating action was also observed if nicotinic acid or aneurin were added to the medium in which the sprout was cultivated.

TABLE VII

Correlation between regeneration of sprouts and development of lateral roots, in the presence of cotyledon-tissue on one side of the embryo. Average of about 20 specimens per group. Results after 8 days' cultivation on standard medium.

Cotyledon-rest		1			½			¼		
Plumule amputated		—	+	+	—	+	+	—	+	+
Regeneration of sprout		—	—	+	—	—	+	—	—	+
Sprout	average length (mm)	121 ± 4.51	—	27±3.50	105 ± 4.26	—	36 ± 2.73	88±4.79	—	27±2.60
	„ fresh weight (mg)	308	—	60	208	—	74	144	—	47
	„ dry weight (mg)	21	—	5	12	—	5	8	—	3
Root with hypocotyl	average length (mm)	100 ± 1.22	40±3.72	93±3.32	91 ± 4.08	64±7.04	102 ± 2.02	66±4.98	59±2.08	97±2.48
	„ fresh weight (mg)	76	57	119	59	83	120	41	70	98
	„ dry weight (mg)	9	6	13	7	10	13	5	8	10
Average number of lateral roots		14.7	0.9	13.6	10.7	4.9	13.6	1.5	2.9	10.7

d. Effect of amputating the top of the sprout (terminal bud) at various stages of development

Under natural circumstances too, it often happens that injury is inflicted upon the plumule, or—at a somewhat further stage—upon the terminal bud, of the seedling. As a rule, regeneration occurs by the development of either an axillary bud of a cotyledon or a lateral bud of the sprout. Now, a study was made of the correlation-phenomena arising on intentional removal of the plumule, or, at a further stage, of the terminal bud.

From peas, 6.0–6.5 mm in diameter, the embryo was isolated and transferred on to the standard medium, containing 0.4 per cent agar. At the very beginning, twenty objects were deprived of their plumule. After 4, 6, 14 and 17 days, resp., the terminal buds were removed from twenty specimens. A number of control groups was left intact (in this respect, for the rest, they lacked the two cotyledons from the start of the experiment).

It appeared that an axillary bud of the cotyledon developed into a new sprout, if the plumule was removed immediately at the beginning. Its growth was somewhat retarded in comparison with a normal sprout; the root, however, was more vigorous. Moreover, the root was distinctly whiter than in the controls; lateral roots were longer and the root-cap had developed more clearly.

If the terminal bud was amputated after 4 or 6 days, part of the sprout was still present, whereas another one developed from one of the axillary buds of the cotyledon. Here too, root growth surpassed that of the controls; the lateral roots had increased both in number and in length and the root-cap had developed well.

If, finally, the terminal bud was removed much later (after 14 or 17 days), a lateral bud usually developed because, meanwhile, the sprout had finished already the first internode. The growth of the main root was not stimulated any longer by this operation.

The results are summarized in table VIII.

§ 2. Early development of the embryo under the influence of various substances

It may be imagined that the correlation between the development of the various parts of the seedling can be interfered with, not only by amputating organs or parts of them, but also by adding one or more substances to the nutrient medium. Previous to studying the subject proper—the influence of growth substances—it seemed advisable to investigate the constituents of the nutrient medium as to their effect at different concentrations. The effects of adding several other organic compounds to the medium and those, following variations of pH were studied as well.

a. The inorganic ingredients of the medium

By varying the concentrations of all the inorganic ingredients (see p. 22) optimal growth was found at the following concentrations:

TABLE VIII

Effect of amputating the top of the sprout upon development of both sprout and root, and upon the number of lateral roots. Average of about 20 specimens. Results after 22 days' culture on standard medium.

		Plumule or terminal bud amputated					
		not at all	at once	after 4 days	after 6 days	after 14 days	after 17 days
Sprout	average length . . . (mm)	72 ± 1.69	61 ± 2.47	61 ± 3.23 *)	57 ± 3.09 *)	57 ± 2.49 *)	60 ± 2.06 *)
	„ fresh weight . (mg)	45	43	50	43	31	34
	„ dry weight . (mg)	3	2	3	3	2	2
Root with hypocotyl	average length . . . (mm)	48 ± 1.18	73 ± 2.83	64 ± 3.07	61 ± 2.72	50 ± 1.47	51 ± 1.69
	„ fresh weight . (mg)	28	44	37	35	27	27
	„ dry weight . (mg)	3	5	5	4	4	3
Average number of lateral roots . . .		3.4	9.9	9.8	11.9	6.1	6.2

*) In these groups the lengths of both the old and the regenerated sprout were put together. The same applies to fresh and dry weights, respectively.

calcium nitrate 3×10^{-3} mol. (708 mg/l), *potassium nitrate* 3×10^{-3} mol. (303 mg/l) and *potassium dihydrogen phosphate* 4×10^{-3} mol. (544 mg/l).

No optimum could be ascertained for either *magnesium sulphate* up to 3×10^{-3} mol. (740 mg/l) or *potassium chloride* up to 6×10^{-3} mol. (448 mg/l).

However, the concentration of *ferric sulphate* proved important, a maximum number of lateral roots being produced at 10^{-4} mol. The same applied to *ferric chloride*.

b. Various sugars

If sugar was absent in the medium, development was but scanty; some elongation of the embryo occurred. The addition of sugar caused growth; however, the optimum concentrations for sprout development and for root growth generally did not coincide. The formation of a root-cap, as well as the maximum number of lateral roots, were dependent on particular concentrations.

Under the circumstances of the present investigation the optimum concentrations for sprout growth, root growth and development of lateral roots, resp., were the following:

dextrose : 3×10^{-1} mol. (54 g/l); 4×10^{-1} mol. (72 g/l); and 2×10^{-1} mol. (36 g/l);
laevulose : 2×10^{-1} mol. (36 g/l); 4 (to 5) $\times 10^{-1}$ mol. (72–90 g/l); and 4×10^{-1} mol. (72 g/l);
sucrose : 2×10^{-1} mol. (68 g/l); 3×10^{-1} mol. (103 g/l); and 4×10^{-1} mol. (137 g/l);
maltose : 1 (to 2) $\times 10^{-1}$ mol. (36–72 g/l); 10^{-1} mol. (36 g/l); and 10^{-1} mol. (36 g/l).

A comparison of these sugars clearly demonstrated their importance in development. It is a matter of specific physiological action, rather than a realization of certain osmotic conditions. This was confirmed once more by testing the effect of combinations of a sugar with *d-mannitol*, which, in itself, is inactive biologically.

So far, the action of *sucrose* seemed to show the largest resemblance to the natural development under the influence of the cotyledons. In all likelihood, in the intact plant as well, sucrose will play an important part in these processes, considering its abundant occurrence in the cotyledons (234).

c. Amino acids

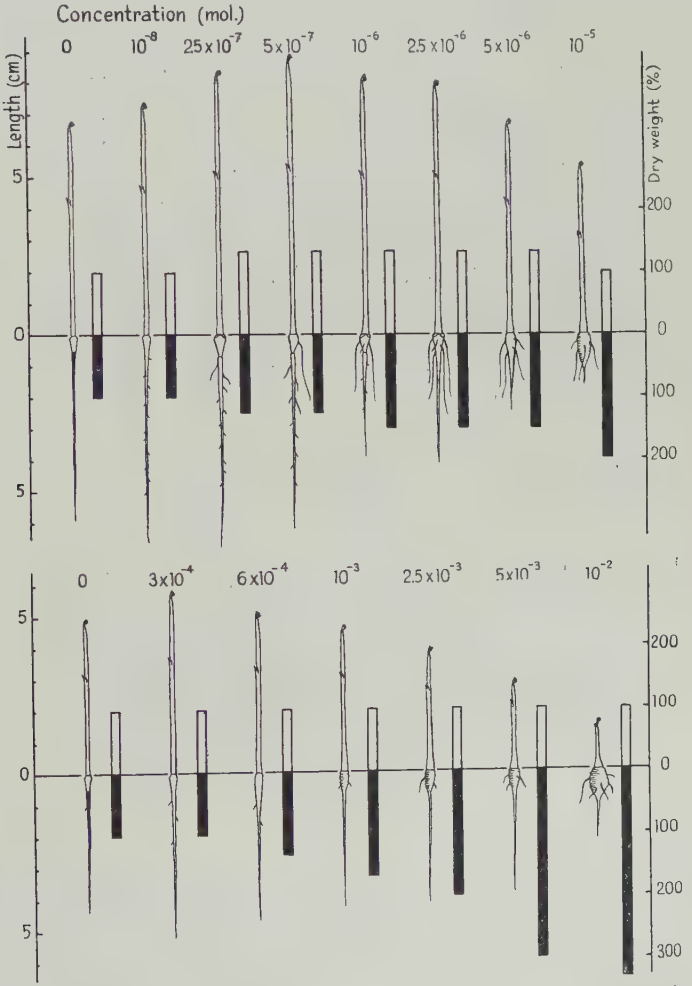
Among the amino acids tested, little or no influence was exerted by *glycine*, *l(+)alanine* and *l(−)asparagine*. *l(—)Aspartic acid* caused some stimulation of growth in sprouts and in roots, at 5×10^{-6} and 5×10^{-4} mol., respectively. From 10^{-3} mol., inhibition revealed itself, which, apart from that, was also observed with glycine at the same concentration.

l(+)Glutamic acid scarcely affected growth, though, at 10^{-7} mol. there was a slight stimulation of the development of lateral roots. Sprout growth was promoted by both *dl-methionine* (10^{-4} mol.) and

l(—)*histidine* (5×10^{-5} mol.). A combination of these two acids acted yet more favourable.

d. Growth substances

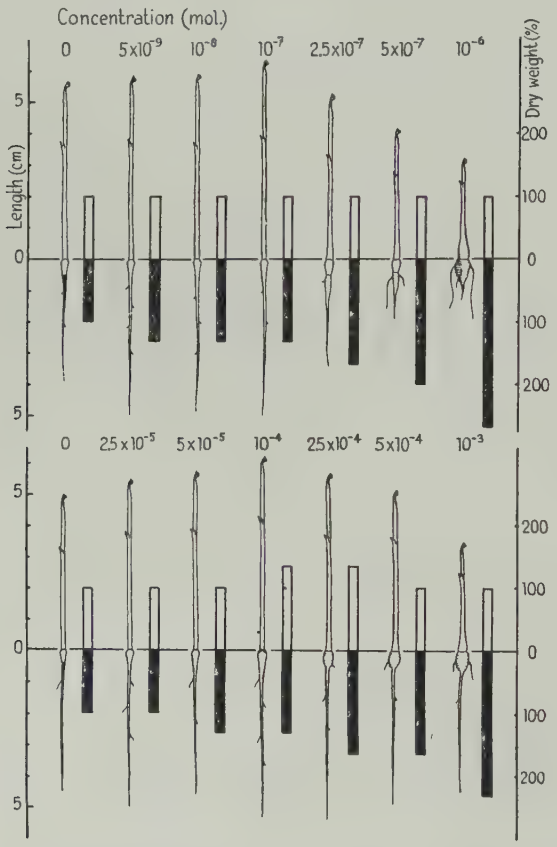
The main object of this investigation is studying the influence of growth substances upon the embryo, after its isolation from the seed, as an attempt at elucidation of the many discrepancies in the results described in the literature.



Figs. 3 and 4. Influence of different concentrations of the potassium-salt of i.a.a. administered via the nutrient medium (above, fig. 3) or during the pre-soaking period (below, fig. 4). The plants pictured show the *development* after three weeks, in proportion to the control-group. Vertical columns represent *dry weights* of both the sprout (above the zero-level) and the root, together with the hypocotyl (below the zero-level). Values in percentage of controls (= 100%). Average of ± 20 specimens.

For this purpose, the potassium salts of *indole-3-acetic acid*, of *naphthalene-1-acetic acid* and of *2,4-dichlorophenoxyacetic acid* were added to the standard nutrient medium, in such a way that concentrations largely diverged.

The phenomena observed proved very specific, and quite different from those produced by other substances. In general, the growth of both sprout and root was stimulated at very low concentrations (i.a.a.K: 10^{-8} mol.; n.a.a.K: 2.5×10^{-8} to 10^{-7} mol.; 2,4-DK:



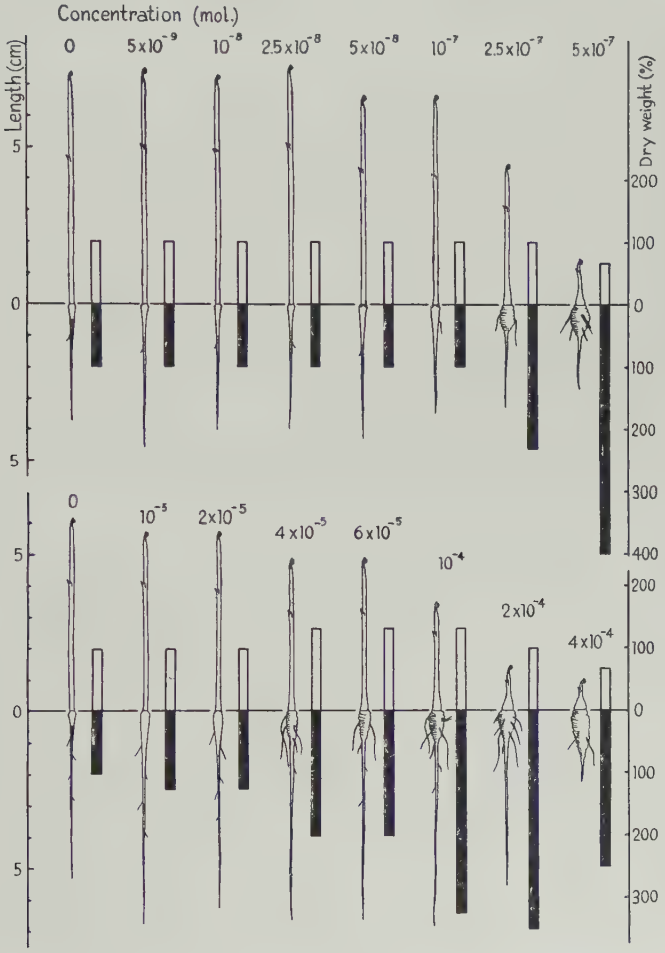
Figs. 5 and 6. Influence of different concentrations of the potassium-salt of n.a.a. administered via the nutrient medium (above, fig. 5) or during pre-soaking period (below, fig. 6). For explanation cf. figs. 3 and 4.

5×10^{-9} to 2.5×10^{-7} mol.). At higher concentrations (from 2.5×10^{-7} mol.) inhibition of growth occurs, accompanied by a swelling of the hypocotyl and by an increase of the amount of lateral roots.

In contradistinction to WHITE's view (247, p. 188) it was noticed that, though the main root was reduced to a short axis, the number of

lateral roots showed an absolute increase by the action of growth substances.

Much importance was attached to the fact that, on increasing concentrations of the growth substance (up to a certain maximum) both the fresh and the dry weights (especially those of the root) kept on rising, in spite of the inhibition of development on the whole. The



Figs. 7 and 8. Influence of different concentrations of the potassium-salt of 2,4-D administered via the nutrient medium (above, fig. 7) or during pre-soaking period (below, fig. 8). For explanation cf. figs. 3 and 4.

boundary line between the hypocotyl and the root finally disappeared, while in that case fasciation of roots frequently occurred. Details are pictured in figures 3, 5 and 7.

It would be worth while tracing the cause of this gain in weight in the presence of a growth substance. Investigations on full-grown

plants, carried out by various authors, revealed an influence upon both the potassium balance and the carbohydrate metabolism.

e. Other organic compounds

Reductone, in general, did not stimulate growth, though at 10^{-3} mol. the longitudinal growth of the lateral roots was promoted, as compared with the controls. From 5×10^{-3} mol. it acted as an inhibitor.

If *d-mannitol* or *inulin* were substituted for sucrose, development failed. The former caused inhibition, if added to a medium with an adequate source of carbon.

Coumarin slightly stimulated sprout growth at 10^{-5} mol. Inhibition was seen from 10^{-4} mol., whereas in roots this occurred already from 2×10^{-5} mol. Up from 5×10^{-5} mol., both the hypocotyl and the root became warty and yellowish.

The potassium salt of *di-n.amyl-acetic acid* inhibited the growth of the sprout (from 5×10^{-6} mol.) and of the root (at 5×10^{-7} mol.). *Adenine* was without any influence.

Adenosine triphosphate (ATP) effected growth stimulation at 7.5×10^{-4} mol., the number of lateral roots being on the increase.

Glutathione somewhat promoted development of lateral roots at 10^{-4} and 2.5×10^{-4} mol.

The addition of (+)*biotin* to the standard medium hardly took any effect, in contrast with the observations by Kögl and Haagen-Smit (236); more lateral roots and some growth-stimulation were not seen below 5×10^{-5} mol.

According to Řetovsky and Horák (246), thiophane-2,5-dicarboxylic acid would equal biotin in stimulating the growth of yeast. Aqueous solutions, as low as 10^{-12} mol., would generate adventitious roots in epicotyls of *Vicia faba*, growth-promotion of isolated plant embryos being reported as well.

This certainly justified its testing on the pea. Both *rac.* (*trans*)-*tetrahydrothiophene-α,α'-dicarboxylic acid* and its *cis-isomer* (10^{-4} mol.) promoted the growth of either sprout and root; lateral roots increased.

Vitamin B_1 favoured sprout growth (3×10^{-7} to 3×10^{-6} mol.) and root growth (1.5×10^{-6} mol.); lateral roots tended to increasing (3×10^{-5} mol.). *Vitamin* B_2 was practically inactive.

Nicotinic acid, scarcely affecting the growth of sprouts and roots, was not quite inactive towards lateral roots (5×10^{-5} mol.).

l-Pantothenic acid proved a weak stimulant for root growth only; from 10^{-5} mol. inhibition took place.

Thus, neither of these substances caused the characteristic phenomena, to be observed after adding a growth substance to the nutrient medium.

f. Effect of pH

The connection between pH and the development of the embryo was studied by adding phosphate buffers (acc. to Sørensen) to the nutrient medium.

When investigating the effect of pH on growth and, possibly, on

certain correlation-phenomena, it should be borne in mind that one never can detect the influence of pH in itself. There are always possibilities of additional influences by certain combinations of salts, or salt concentrations, or by changes in the viscosity of the agar.

From pH 7.2 upwards, decomposition of sucrose occurred on sterilizing. A distinct optimum was found at pH 5.1, for growth (of both the sprout and the root) as well as for the formation of lateral roots. Below pH 5.1 development was satisfactory, but growth declined. From pH 5.8 upwards, development was inhibited more and more.

Summary

It was supposed that on treating seeds with a growth substance, its influence upon the embryo was exerted not only directly, but also indirectly, via the cotyledons. Therefore it was studied first, what influence might be exercised on early development by the two cotyledons and by parts of them.

Correlation-phenomena occurring normally were examined, as well as those occurring after amputation of cotyledons and those, observed on regeneration, after the terminal bud had been amputated.

The addition of a growth substance to the nutrient medium produced a very specific effect on the development of the embryo. By varying the concentrations of all the ingredients of the medium and by testing the effect of several organic compounds, it was ascertained that their action can be distinguished clearly from that of the growth substances.

Finally the effect of pH on the development of the embryo was studied.

Chapter V

ACTION OF GROWTH SUBSTANCES ON THE EMBRYO, DIRECT AS WELL AS INDIRECT (VIA THE COTYLEDONS)

§ 1. The action of growth substances in seed-treatment

Some authors have moved certain points that should be considered in seed-treatment, the more so as they might be related to varying results (compare Chapter I). Beside these points, however, there seem to be a few other ones, deserving more attention than they have received before. The following items are worth while considering:

1. Both size and origin (viz., location in a multispermous fruit) and ripeness may differ in a given lot of seed.
2. The selective action of the seed-coat may differ as well, in dependence on the foregoing. As a semi-permeable membrane it will make difference when substances are passing through, towards the embryo. Thus, the way in which the growth substance is administered —e.g. as such, or as a salt—may play some part.
3. Too little allowance is made for the fact that the growth substance acts not only direct on the embryo, but also by some indirect way. For, after being absorbed at the swelling of the cotyledons, it may keep

exerting an influence during the development of the seedling. In that case the cotyledon would act like a reservoir, gradually giving off its contents.

Ad. 1. The connection between size and subsequent development of the seed was already outlined in Chapter III, § 1. It will be shown below that this point may be important in growth substance treatment too.

Ad. 2. In the object chosen, semi-permeability of the seed-coat probably will play little part, which was already demonstrated by VAN DER MAREL (241)—in contrast to the seeds of, e.g., Cucurbitaceae. For the matter of growth substances, however, nothing has been proved so far.

The influencing of water uptake during the earlier phases of germination (viz., swelling) by growth substance treatment was tentatively studied (see Chapter VI).

Ad. 3. If such an indirect influence should prove a general phenomenon in seed-treatment, different results might be expected if the same growth substance (even of the same concentration) would be applied to seeds, varying in the nature of the reserve substances (e.g. carbohydrates, proteins, or lipids) present in their cotyledons. In that case, small variations in growth substance concentrations, together with differences both in the size of the seeds and in the proportion of embryo-size to volume of reserve substances, might lead to variability of results.

The extent of the above-said, indirect influence was approximated in three ways:

by comparing the development of embryos from seeds pre-soaked in water (on a medium containing growth substance) with those from seeds pre-soaked in growth substance solutions (cultivated on a standard medium, i.e., without growth substances): § 2;

by studying the development of embryos with different quantities of reserve food, isolated from seeds, pre-soaked either in water or in growth substance solutions: § 3;

by estimating the development of embryos from seeds, pre-soaked either in water or in growth substance solutions, on a medium without growth substances, after the cotyledons had been amputated at various stages: § 4.

§ 2. COMPARISON OF THE DEVELOPMENT OF EMBRYOS FROM SEEDS, PRE-SOAKED IN WATER OR IN GROWTH SUBSTANCE SOLUTIONS, ON NUTRIENT MEDIA WITH OR WITHOUT GROWTH SUBSTANCES, RESPECTIVELY

The influence of the three growth substances, tested in the present investigation (i.a.a., n.a.a. and 2,4-D), was mentioned before (p. 39–42); it proved most characteristic (figs. 3, 5, 7).

The action of growth substances, administered during pre-soaking only, was studied under absolutely sterile conditions by adding 20 ml of their solutions (of various concentrations) to peas placed in Petri dishes. After soaking for some 16 hours, in the usual way at 24° C

in the dark, the seeds were carefully rinsed three times with sterile, distilled water. Any interference by residual growth substance thus being avoided, the isolated embryos were transferred into the culture tubes.

a. Pre-soaking in potassium indole-3-acetate

The experiment was terminated after three weeks (fig. 4). Treatment with a 3×10^{-4} mol. solution had stimulated the longitudinal growth of both sprout and root. The result after this short period of cultivation comes up to expectations of seed-treatment.

At higher concentrations growth was inhibited, though fresh and dry weights of the root kept increasing. From 6×10^{-4} mol. onwards the influence of the growth substance clearly manifested itself by an increasing number of specimens, the hypocotyl of which got swollen. The number of lateral roots augmented, and attained its maximum at 10^{-2} mol. i.a.a.K.

The boundary line between the white hypocotyl and the dark root, clearly visible at first, became less distinct from 10^{-3} mol. onwards because the hypocotyl got brown as well. Besides, swelling occurred at the basal part of the sprout.

Generally speaking, the above phenomena are in keeping with the observations made during the culture of embryos on nutrient media, containing various amounts of i.a.a.K (see p. 39 and fig. 3). However, the concentration required for a similar effect is many times higher if the growth substance is applied during pre-soaking; quite obvious, because the administration during a sixteen hours' soaking period is quite different from that during a three weeks' culture.

b. Pre-soaking in potassium naphthalene-1-acetate

The results of an experiment with n.a.a.K are summarized in fig. 6. In general, they resemble those obtained after pre-soaking in i.a.a.K. An increase of longitudinal growth of sprout and root was seen only at higher concentrations (from 2.5×10^{-5} to 10^{-4} mol.) Subsequently both organs showed shrinking, whereas fresh and dry weights of the root, including the hypocotyl, kept rising.

The hypocotyl, from 10^{-4} mol. onwards, started swelling and it became more and more callous at increasing concentrations. The basal part of the sprout became swollen too, from 5×10^{-4} mol. onwards. The average number of lateral roots attained its maximum at 10^{-4} mol.

A comparison of the results with those obtained with nutrient media containing n.a.a.K (p. 40, fig. 5) shows their similarity.

c. Pre-soaking in potassium 2,4-dichlorophenoxyacetate

Stimulation of sprout growth did not appear at the concentrations tested (fig. 8). Fresh and dry weights, however, were raised; the optimum being attained at 4×10^{-5} to 10^{-4} mol.

Root growth was distinctly favoured between 10^{-5} and 10^{-4} mol. Optimum conditions for fresh and dry weights were found at 2×10^{-4}

mol. Again it was chiefly the hypocotyl that became swollen at the lowest dose applied; from 4×10^{-5} mol. onwards it turned brown, so that the boundary line with the root proper got lost.

Pre-soaking in this growth substance raised the number of lateral roots and, from 4×10^{-5} mol. onwards, the larger part of them was found implanted on the swollen hypocotyl. The lateral roots had grown longer than those of specimens, pre-soaked in plain water; now and then fasciation of roots was observed.

Just as with i.a.a.K and n.a.a.K, the effect of 2,4-DK, applied during pre-soaking, is generally comparable to that, produced via the nutrient medium (comp. the data in figs. 7 and 8).

So, it may be concluded that the effect of a treatment with these three growth substances during pre-soaking is fairly comparable to the influence exerted on the isolated embryo, via the nutrient medium. In the former case, however, a similar effect is only obtained at much higher concentrations.

Most striking is the influence of the growth substances upon root development. Low concentrations stimulate longitudinal growth, higher doses cause inhibition, whereas fresh and dry weights keep increasing.

It should be noted that the inhibitory influence upon root growth is less radical if the growth substances are applied during pre-soaking.

TABLE IX

Comparison of optimum concentrations of growth substances, applied to peas, 6.0 — 6.5 mm in diameter

	i.a.a.K	n.a.a.K	2,4-DK
Action on the embryo, via nutrient medium:			
promotion of sprout growth	10^{-8} mol.	10^{-7} mol.	5×10^{-9} mol.
promotion of root growth	10^{-8} „	2.5×10^{-8} „	5×10^{-9} „
incipient inhibition of sprout growth	10^{-7} „	2.5×10^{-7} „	5×10^{-8} „
incipient inhibition of root growth	2.5×10^{-7} „	2.5×10^{-7} „	10^{-7} „
swelling of hypocotyl	5×10^{-7} „	2.5×10^{-7} „	2.5×10^{-7} „
distinct inhibition, all over	5×10^{-7} „	2.5×10^{-7} „	2.5×10^{-7} „
maximum number of lateral roots	10^{-7} „	6×10^{-6} „	5×10^{-7} „
Action after soaking the seed, via cotyledons:			
promotion of sprout growth	$\pm 3 \times 10^{-4}$ mol.	$\pm 10^{-4}$ mol.	?
promotion of root growth	$\pm 3 \times 10^{-4}$ „	$\pm 10^{-4}$ „	$\pm 10^{-4}$ mol.
incipient inhibition of sprout growth	$\pm 10^{-3}$ „	$\pm 9 \times 10^{-4}$ „	$\pm 10^{-5}$ „
incipient inhibition of root growth	$\pm 10^{-3}$ „	$\pm 9 \times 10^{-4}$ „	$\pm 3 \times 10^{-4}$ „
swelling of hypocotyl	$\pm 10^{-3}$ „	$\pm 10^{-4}$ „	$\pm 2 \times 10^{-5}$ „
distinct inhibition, all over	$\pm 10^{-3}$ „	$\pm 9 \times 10^{-4}$ „	$\pm 10^{-4}$ „
maximum number of lateral roots	$\pm 10^{-2}$ „	$\pm 10^{-4}$ „	$\pm 10^{-4}$ „

It is distinctly visible (figs. 3 and 4, 5 and 6, 7 and 8) that swelling is restricted to the original junctures of the cotyledons.

Table IX summarizes the optimum concentrations of the growth substances for certain, discernible phenomena, appearing after administering in both ways.

The figures show that, on the whole, 2,4-DK is the most active agent; the optimum concentrations of n.a.a.K are higher and those of i.a.a.K even a little more.

For each effect recorded, one can calculate the proportion of the concentrations required in direct and in indirect application of each of these substances. Now, if attention is paid to a very specific effect (e.g., the swelling of the hypocotyls), considerable differences prove to exist (table X).

TABLE X

Approximate factors, obtained by dividing the growth substance concentrations required in action via the cotyledons (during pre-soaking) by those required in direct action (via the nutrient medium), for a similar final result.

	i.a.a.K	n.a.a.K	2,4-DK
Promotion of sprout growth	30.000	1.100	?
Promotion of root growth	30.000	4.000	20.000
Incipient inhibition of sprout growth .	10.000	3.500	200
Incipient inhibition of root growth . .	5.000	3.500	3.000
Swelling of hypocotyl	2.300	400	80
Distinct inhibition, all over	2.300	3.500	400
Maximum number of lateral roots . .	100	18	200

Though there is certainly a difference between continuous contact with a growth substance solution and a relatively short administration of growth substance through the seed-coat, there are qualitative features in the reaction that cannot be accounted for by this difference.

These can only be accounted for by presuming that, during the action via the cotyledons, some interaction with other factors has occurred (selective action of the seed-coat, influence of constituents of the cotyledons). However, one should not overlook the fact that i.a.a., for instance, is poorly stable; it might be decomposed in some enzymatic process.

For the sake of comparison, peas were also soaked in *coumarin* solutions. Cultivation of the embryo on a medium containing this inhibitory substance led to restriction of growth, in both the sprout and the root, at concentrations from 10^{-4} mol. upwards (cf. p. 42). Though the concentration was raised to 7×10^{-4} mol. (being the maximum solubility in water) no other effects were seen. The inhibitory action of such coumarin solutions upon the germination of whole peas was not very striking either; only root growth was slightly reduced. In comparison with other types of seeds (e.g., those of the garden cress) the pea is evidently but little sensitive to this substance. It is possibly a question of enzymatic decomposition in the cotyledons, and these findings might be an argument in support of the author's view

that in seed-treatment the substances administered would exercise their influence mainly via the cotyledons.

§ 3. Study on the development of embryos with different quantities of reserve food, isolated from seeds, pre-soaked either in water or in a growth substance solution

It was already stated (p. 30) that the cotyledon-tissue exerts an enormous influence upon the development of the embryo and upon the formation of lateral roots. If one imagines the simplest case, viz. that during pre-soaking in a growth substance solution the cotyledons initially absorb the growth substance and that they pass it on—as such—to the embryo afterwards, an inhibitory influence should increase at a suitable concentration the more cotyledon-tissue is left to the embryo.

After sterilizing, peas of one size (6.0–6.5 mm in diameter) were pre-soaked in water or in sterile solutions of a growth substance, at about 24° C in the dark. Some 16 hours after, the seeds were washed three times with sterile, distilled water and subjected to excision of either embryos or embryos to which varying amounts of cotyledon-tissue had been left (1/4, 2 × 1/4, 1/2, 2 × 1/2, 1 and 2 × 1 cotyledon). Cultivation on the standard nutrient medium took place in the dark at about 24° C. After ten days the test was finished.

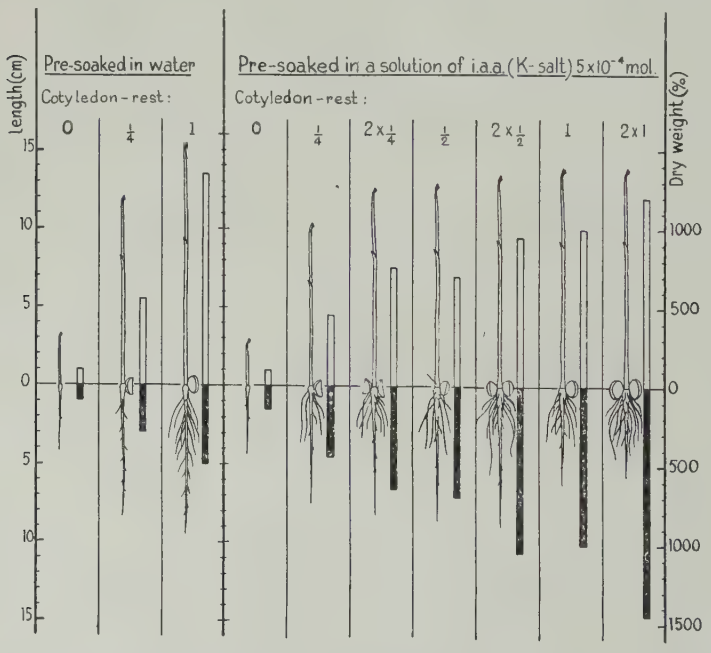


Fig. 9. Development of pea-embryos with different quantities of reserve-food, after the seeds had been pre-soaked in water or in a solution of i.a.a. (K-salt) 5×10^{-4} mol. Results after ten days. For explanation cf. figs. 3 and 4.

a. Pre-soaking in potassium indole-3-acetate

No doubt the concentration chosen for an initial experiment is preferably such as to produce scarcely any effect in cultivating the embryo (i.e. without reserve food). To begin with, a 5×10^{-4} mol. solution of i.a.a.K was used (fig. 9).

Undesired cotyledon-tissue was cut off at a rough estimation (cf. fig. 2, C), and subsequently weighed in the fresh state and after drying. When the test was over, fresh and dry weights of the cotyledon-parts remained could be determined, which revealed the amounts of tissue, having taken part in the growth process.

The development after pre-soaking in water has been outlined already. A small amount of cotyledon-tissue stimulates the growth of sprout and root to the extent that after the ten days' period the majority of the specimens has grown against the cotton plug, the lower parts being pressed down into the agar. Liquefaction of the medium ensues from this. The main roots have a dark hue, lateral roots have developed either as tiny points, or as roots up to 30 mm in length; the root-cap is either hardly visible, or not at all.

The presence of one cotyledon promotes growth still more, as well as the number of lateral roots; the root-cap is clearly visible as a brown tip.

The action of the growth substance answered expectations to a small degree only. As to longitudinal growth, the influence on the embryo is very slight indeed. *In the presence of cotyledon-tissue, however, there is always stimulation of growth.* In comparison with the corresponding, non-treated lots some growth inhibition of sprout and root was noticed, it is true; nevertheless, fresh and dry weights of the root increased in consequence of the treatment (fig. 9). Now, quite remarkable, the lateral roots were implanted chiefly on the swollen hypocotyl and on the upper part of the root.

Another feature was, that two quarters apparently effected the same as half a cotyledon and, likewise, the influence of two halves equalled that of a whole cotyledon. No doubt the differences observed may be ascribed to the circumstance that these amputations were made at random.

The results of another trial with i.a.a.K, at a higher level (10^{-3} mol.) corroborated these findings. Raising the concentration clearly enhanced the inhibition of longitudinal growth of sprout and root; despite of this, fresh and dry weights of the root were on the increase again.

In comparison with the corresponding water controls, the increase of root weight is the larger, the more reserve substance tissue is left to the embryo.

b. Pre-soaking in potassium naphthalene-1-acetate

The results of a ten days' test with n.a.a.K (5×10^{-5} mol.) showed much resemblance to those obtained with 5×10^{-4} mol. i.a.a.K

(fig. 10). On closer examination, however, this does not apply to the inhibition: the combination of reserve substance and n.a.a.K (the dose of which was only one tenth of that in the first test with i.a.a.K) had caused less inhibition of sprout and root. Again, in the presence of more reserve food tissue, the main effect of the growth substance was an augmentation of fresh and dry weights of the roots.

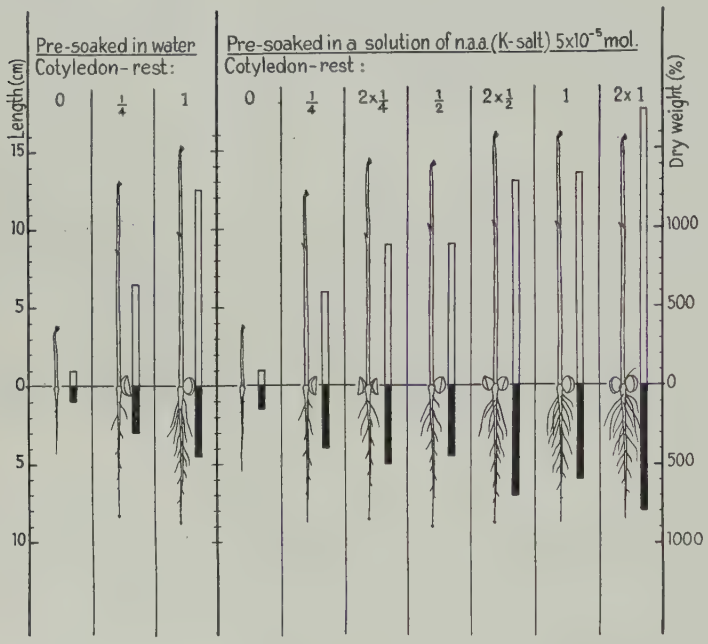


Fig. 10. Development of pea-embryos with different quantities of reserve-food, after the seeds had been pre-soaked in water or in a solution of n.a.a. (K-salt) 5×10^{-5} mol. Results after ten days. For explanation cf. figs. 3 and 4.

c. Pre-soaking in potassium 2,4-dichlorophenoxyacetate

A comparatively low concentration of 2,4-D, viz. 2.5×10^{-6} mol., was much more effective than the two substances mentioned above (fig. 11). Though, in the absence of reserve food hardly any inhibition took place, it was distinctly visible when, after the treatment, embryos with one quarter or a whole cotyledon were compared with the water controls.

The competition of growth promotion by the cotyledon-mass with inhibition by the growth substance was mostly won by the latter. In some specimens from the groups with two quarters, or more, of a cotyledon, inversion of polarity was observed. At first, the sprout grew downward, then it became negatively geotropic. The callous, swollen root-stump pointed upwards, rising above the medium.

Just as in all the previous experiments the treatment strongly affected both hypocotyl and root. An increase of fresh and dry weights was the final result.

A concentration four times higher (10^{-5} mol. 2,4-DK) produced quite another picture. After ten days the embryo without reserve substance was clearly inhibited already; the basal part of the sprout had thickened and the hypocotyl had swollen. In the presence of cotyledon-tissue, inhibition was very strong; here, in comparison with the two other substances, the action was rather a toxic one. Most of

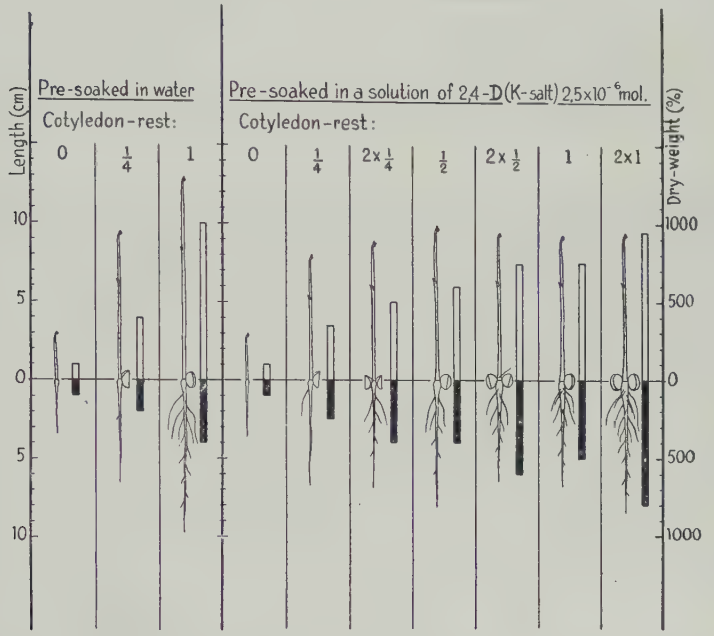


Fig. 11. Development of pea-embryos with different quantities of reserve-food, after the seeds had been pre-soaked in water or in a solution of 2,4-D (K-salt) 2.5×10^{-6} mol. Results after nine days. For explanation cf. figs. 3 and 4.

the specimens had grown into the agar medium sprout downwards, the callous root-mass aloft. Despite of the strong inhibition of longitudinal growth in sprout and root, a considerable increase of fresh and dry weights never failed with the root, including the hypocotyl.

When culture at this high concentration was protracted for over ten days, the sprouts of several specimens were still capable of growing further.

The experiments might be carried out with peas of different size, provided some changes are made. For, in the pea, no mean differences are found in the proportion of embryo-mass to reserve substance, in comparing seeds of unequal size (cf. p. 24). In that case, however, mutual comparison is hampered by disparity of the embryo.

For a clear apprehension of the growth substance action this investigation ought to be extended to tests in which, during pre-

soaking, such concentrations are applied as to finally procure equal amounts of growth substance for the embryos of all groups, after partial amputations of cotyledon-tissue.

The following series of tests was set up:

	Peas, pre-soaked in:	Number of cotyledons, left to embryos under cultivation:
A	water (controls)	0, 1/4, 1
B	i.a.a.K. 4×10^{-3} mol.	0, 1/4
C	„ 2×10^{-3} „	0, $2 \times 1/4$, 1/2
D	„ 10^{-3} „	0, $2 \times 1/2$, 1
E	„ 5×10^{-4} „	0, 2×1

Barring the lots, consisting of embryos without reserve substance tissue, the concentrations were chosen in such a way as to halve the dose of growth substance on doubling the quantity of cotyledon-tissue. Assuming that the growth substance is uniformly absorbed into the cotyledons and that its action is proportional to the mass of reserve substance tissue, ultimately about the same dose should be present in the following combinations:

1/4 cotyledon and i.a.a.K 4×10^{-3} mol.; $2 \times 1/4$, or 1/2 cotyledon and 2×10^{-3} mol.; $2 \times 1/2$, or 1 cotyledon and 10^{-3} mol., and, finally, 2×1 cotyledon and i.a.a.K 5×10^{-4} mol.

Judging from the results of earlier experiments, expectations were not put too high in this case either. The lower the growth substance concentration, the less the embryo (without cotyledons) is inhibited, of course. This was already shown in the earlier pre-soaking tests with i.a.a.K (fig. 4).

The highest concentration, 4×10^{-3} mol., strongly inhibited the development of the embryo with one quarter of a cotyledon, though to a lesser degree than that of the plain embryo. Nearly all of the embryos had turned upside down, i.e., the sprout had penetrated into the agar, whereas the root had grown upwards. Both of the organs bent at their ends finally, thus striving after re-establishment of the polarity. *The less growth substance was administered and the more cotyledon-tissue was left to the embryo, the more growth improved.*

Almost every growth substance treatment led to swelling of the hypocotyls and to accumulation of lateral roots, especially on this part of the plant.

From all these tests the following combinations are available for comparison:

I. embryos with equal quantities of cotyledon-tissue and varied concentrations of growth substances (p. 44);
II. embryos with equal quantities of growth substance administered and variable cotyledon-mass (p. 48);
III. embryos with varied quantities of cotyledon-tissue, together with different doses of growth substances, in such a way as to yield about the same total in every case (p. 52).

It should be borne in mind, however, that the action of the growth substance during pre-soaking is twofold. There is a direct action on the embryo by the permeating growth substance that will increase with increasing concentrations. On the other hand growth substances are absorbed by the cotyledons and, afterwards, exert their influence through these.

It is difficult to form a clear-cut picture of the growth substance action proper, the growth stimulation through the cotyledons interfering with the processes involved in the former. How far the nature of the reserve substances is concerned with the problem, cannot be settled before other types of seeds have been tested, the reserve tissues of which consists mainly of lipids or proteins.

§ 4. Comparison of the development of embryos from seeds, pre-soaked either in water or in growth substance solutions, after the cotyledons had been amputated at various stages

Amputation of the cotyledons at various stages (cf. p. 32) has shown that the initial growth process is already considerably favoured if these organs remain connected with the embryo for 1 × 24 hours. After pre-soaking in water or in a growth substance solution, in the dark at about 24° C, the peas were washed three times with sterile, distilled water. The embryos (without cotyledons), from twenty water controls and from twenty peas pre-soaked in the growth substance solution, were transferred to the standard nutrient medium. Two more series were set up, consisting of 5 × 20 peas each, from which only the seed-coats had been removed, after pre-soaking in water or in the growth substance solution, respectively. These embryos with cotyledons were also put into tubes, containing the same medium. Hereafter, they were cultivated in the dark at 24° C, just as the others.

From a water control group and from a treated group, of 20 specimens each, the cotyledons were amputated under aseptic conditions at 24 hours' intervals. From each series a group of 20 specimens was left intact (embryos with cotyledons). The experiment was finished after ten days.

a. Pre-soaking in potassium indole-3-acetate

In the first test with i.a.a.K a 3×10^{-4} mol. concentration was chosen. The result is found in fig. 12: A, B. As to the *water controls*, again the development of both the sprout and the root was favoured during the first 24 hours, reckoning from the moment pre-soaking was concluded. Development improved and lateral roots increased both in number and in length, as the cotyledons remained attached for a longer time. At a certain stage the development of the root-cap became manifest. Moreover, in the groups 3 to 6, inclusive, the agar liquefied.

After pre-soaking in the growth substance solution some inhibition was seen in the development of the embryo without reserve substance. The same effect was observed in young plants, to which the cotyledons had been left for a longer time. Amputation after 2 × 24 hours resulted

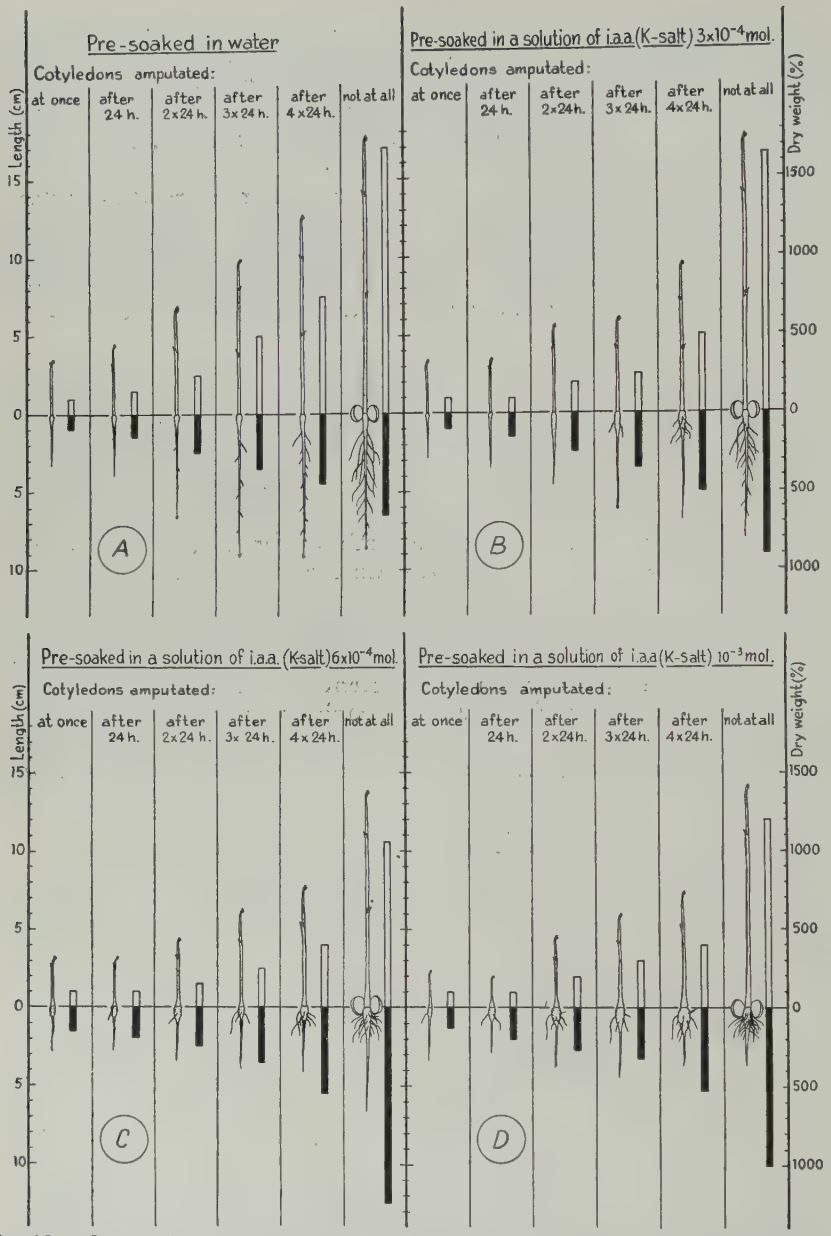


Fig. 12. Comparison of the development of pea-embryos from seeds pre-soaked in water or in a solution of i.a.a. (K-salt) after the cotyledons had been amputated at different stages after pre-soaking.

A. water-control groups (controls of B);
B. influence of i.a.a.K 3×10^{-4} mol.;
C. „ „ „ 6×10^{-4} mol.;
D. „ „ „ 10^{-3} mol.

Results after ten days. For explanation cf. figs. 3 and 4. The data of C and D are reduced to the relative water-controls omitted in the figure.

in roots slightly thicker than in the water controls, when the test was finished. The next groups showed the picture, typical of growth substance action, viz. swelling of the hypocotyl, and, on to it, the location of lateral roots. Although, in the two remaining groups, growth inhibition of sprout and root continued, the average fresh and dry weights of the root had risen.

Exclusive of the last group, the number of lateral roots had been diminished by the growth substance treatment. Sometimes root fasciation was found.

Another experiment was made with double the concentration of i.a.a.K, so 6×10^{-4} mol. (fig. 12, C). As compared with the preceding test, inhibition by the growth substance was stronger. It was evident already in the instance of the young plant, deprived of its cotyledons at the very beginning. Sprout growth was hampered in particular; moreover, a callous thickening of the hypocotyl was seen. Fresh and dry weights of the root had increased.

Development would slightly improve as the cotyledons were left to the plant for a longer time, though as a whole it was considerably inhibited in comparison with the water controls. In the hypocotyl the treatment, as it were, has caused an accumulation of substances necessary to the development of lateral roots, hence they will develop mainly in this region. Here, too, root fasciation was frequent.

A dose of i.a.a.K even higher (10^{-3} mol.) had chiefly increased the inhibition of root growth (fig. 12, D). It looked as if the working hypothesis would become realized, viz. that the inhibitory action by the growth substance would be the stronger, the longer the cotyledons remain attached. However, amputation after 2×24 hours again resulted in growth stimulation in respect of the preceding group. There was an all-round rise of fresh and dry weights in the root-system including the hypocotyl. The lateral roots, in comparison with the controls, had diminished in number and, as to the groups with prolonged retention of the cotyledons, also in length. The crowding together of a large number of lateral roots in a small area, probably favours root fasciation, which was often observed.

b. Pre-soaking in potassium naphthalene-1-acetate

The influence of n.a.a.K was studied at a concentration of 5×10^{-4} mol. (fig. 13). The overall picture resembled that produced by i.a.a.K, yet, the action was much stronger than that exercised by the more concentrated solution of the latter (10^{-3} mol.). The plant, grown from an embryo isolated at the beginning of the test, shows a hypocotyl amply swollen. Whereas the root, together with the hypocotyl, was shorter than the corresponding part in the control, its dry weight had trebled.

When the cotyledons were amputated later on, inhibition kept going on at first, thus coming up to expectations. Thereupon, however, the beneficial influence of the cotyledons themselves obviously surpassed the effect, so that the longitudinal growth of sprout and root continued

anew. Nevertheless, both organs were inhibited in comparison with the water controls.

The treatment had lowered the number of lateral roots; the tight packing on the hypocotyl led to fasciation.

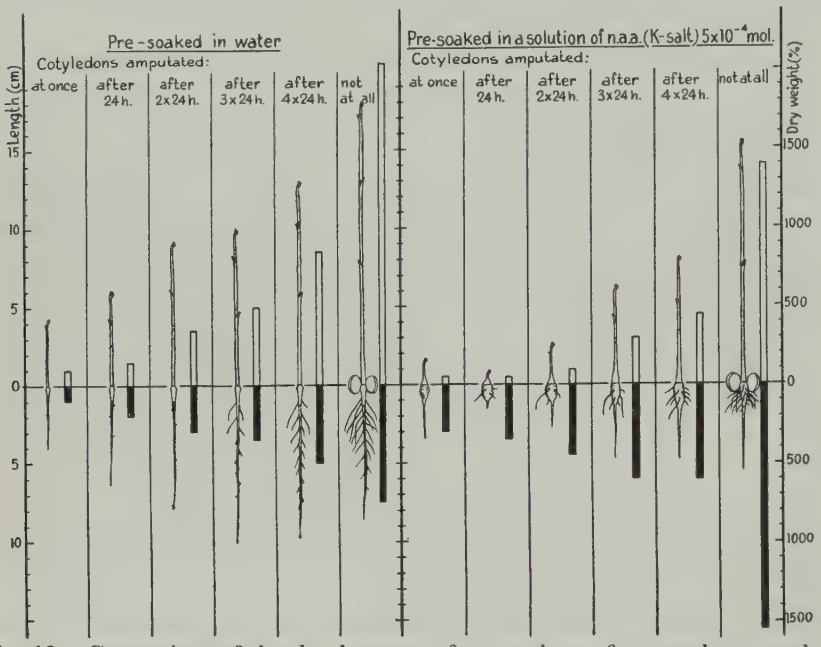


Fig. 13. Comparison of the development of pea-embryos from seeds pre-soaked in water or in a solution of n.a.a. (K-salt) 5×10^{-4} mol. after the cotyledons had been amputated at different stages after pre-soaking. Results after ten days. For explanation cf. figs. 3 and 4.

c. Pre-soaking in potassium 2,4-dichlorophenoxyacetate

The results of an experiment with 2,4-DK, at a concentration of 10^{-5} mol., are summarized in fig. 14. Roughly speaking, the issue was like the situation to be imagined in the event of a simple passing on to the embryo of the growth substance absorbed by the cotyledons, without any growth-stimulating action of their own coming into play. The divergence from the other results described in this paragraph probably must be explained by a toxic action, interfering with the normal, enzymatic processes.

In the water controls, the longer the cotyledons remained attached, the more growth increased, both of sprout and root. *In the growth substance groups, it was just the reverse: development was inhibited more and more, when amputations were done at a later stage.*

The final group, in which no amputation was applied, differed somewhat from the others, in that the growth-promoting influence of the cotyledons was predominant, so that sprout and root would develop a little further.

A comparison of the water controls with the corresponding groups from the growth substance series (mainly those with i.a.a.K) leads to the following conclusions:

1. Longitudinal growth, both of the sprout and the root, is inhibited the stronger, the more the growth substance concentration is raised; at the same time fresh and dry weights of the sprout *diminish*, whereas those of the root (with the hypocotyl) *increase*.

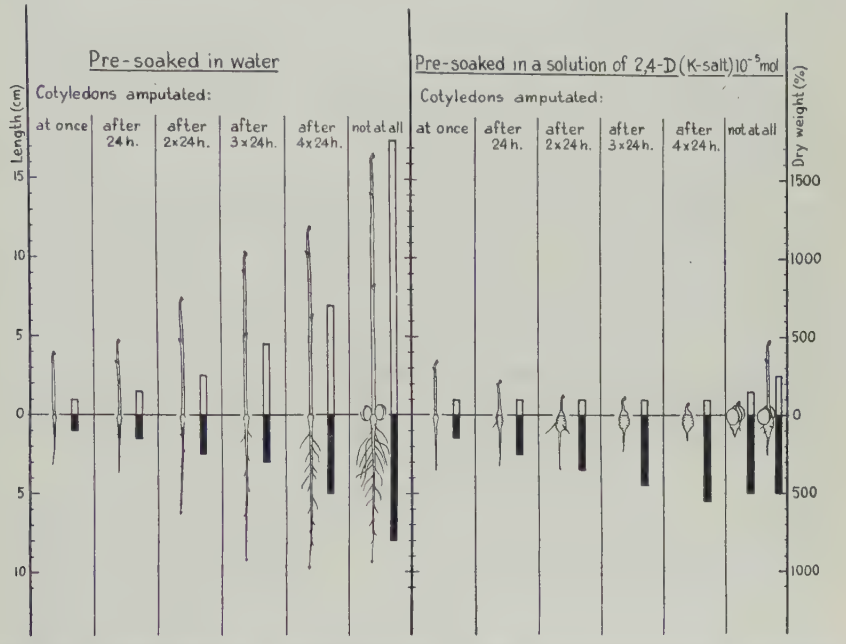


Fig. 14. Comparison of the development of pea-embryos from seeds pre-soaked in water or in a solution of 2,4-D (K-salt) 10^{-5} mol. after the cotyledons had been amputated at different stages after pre-soaking. Results after ten days. For explanation cf. figs. 3 and 4.

2. As the growth substance concentration becomes higher, the period during which the cotyledons remain connected with the embryo will have to be reduced ever more, in order to attain a maximum inhibition of *sprout* growth. The *root* is not affected to such an extent: the strongest inhibition is usually found with the groups in which amputating of cotyledons was done after some 3×24 hours. This is probably linked up with the fact that, in the water controls, maximum longitudinal growth is already achieved after a 3×24 hours' connection (cf. fig. 12, A and table VI).

3. The point of time at which cotyledons should be amputated in order to ultimately raise fresh and dry weights of hypocotyl plus root *above* those of the controls, falls the earlier, the higher the concentration applied.

4. From 2 and 3 it follows that the effect of a high dose of growth substance is passed on to the embryo sooner than that of a low one.

5. At similar concentrations the influence of n.a.a.K and 2,4-DK is stronger than that of i.a.a.K.

Summary

The effect of the three growth substances mentioned above, administered during pre-soaking, is largely comparable to that exercised on the isolated embryo, by way of the nutrient medium.

From the ratio of the optimum concentrations required for certain, striking phenomena, induced either via the medium or via the cotyledons, it might be concluded that, in the latter instance, an interaction with other factors has taken place (selective action of the seed-coat, influence of constituents of the cotyledons).

From tests with different concentrations of i.a.a.K, n.a.a.K and 2,4-DK it appears that the growth substance acts not only directly on the embryo, but also in an indirect way, via the reserve substance mass. The latter influence also manifests itself afterwards.

The growth substance taken up by the cotyledons is passed on to the embryo, as it were, at a gush. Thus, at an appropriate growth substance concentration, amputation of the cotyledons after 1×24 hours (or, sometimes, after a longer time) will cause merely inhibition. The action of the cotyledons, therefore, should not be compared to that of a reservoir, gradually giving off the growth substance to the embryo.

Removing the reserve substance mass at a later stage usually results in a predominance of the growth-stimulating action of the cotyledons, though, in comparison with the corresponding water controls, not without some inhibition. Both sprout and root develop further, which is accompanied by an action typical of growth substances, viz. the lateral roots being crowded together on the callous, swollen hypocotyl.

In general it may be observed that, in spite of the inhibitory influence exerted on the region below the insertion of the cotyledons, the fresh and dry weights of the root (including the hypocotyl) have considerably increased in consequence of the treatment.

A growth substance dose not too high will not cause any serious loss of the total amount of dry matter, but merely a different distribution.

Chapter VI

PRELIMINARY EXPERIMENTS ON WATER ABSORPTION IN SEEDS AND ITS AFFECTION BY A GROWTH SUBSTANCE

§ 1. Introduction

Provided its internal conditions and the temperature are suitable, a seed brought into contact with water will absorb it in a measure. At first the absorption may be largely, or entirely, due to imbibition; as more water is absorbed, the consequent production of osmotically active substances from the reserves may promote the osmotic absorption.

It is within the range of possibility that the initial process is influenced

on treating the seeds with growth substances. The seed-coat, as a *selectively permeable membrane*, may or may not play a part in that case.

Van der Marel (241), in 1919, demonstrated that, as to the pea, the seed-coat is *not* selectively permeable, aqueous solutions passing freely through it. The solute then might influence the embryo quite unhampered.

Interesting data on swelling during the water uptake of the seed are to be found in the papers by Pringsheim (229), Brown (219) and Eyster (220). In several cases the technique applied, from modern standards, was rather lacking in perfection, however. As, for instance, the duration of the experiments gave rise to bacterial contamination, the interpretation of the results was not unambiguous. So, a sensitive and more accurate registration-method might give better information about these processes. Especially the examination of the response of swelling seeds to growth substances was deemed of interest.

§ 2. Water absorption in normal seeds

The water uptake was studied with the aid of small (15-ml) flasks, connected with a glass capillary (1 mm wide and some 400 mm long) by means of a ground joint. The requisite number of seeds, after weighing, was put into a flask which, thereupon, was made up with distilled water, care being taken to expel any adhering air bubbles by shaking. The capillary, greased with a little Apiezon-L for the sake of a tight fitting, was placed on to the flask, while its other end was provided with a piece of para rubber tube.

Eventually, a number of flasks thus filled was ranged horizontally on a frame, each capillary alongside of a rule. The whole was put into a water-filled thermostat, the rubber tubes allowing the capillaries a free communication with the air.

All the materials, the air-dried seeds as well, were conditioned at a temperature of 26° C for some 24 hours, in the same room to be used for the observations. The process of swelling was judged by the displacement of the meniscus in the capillary. Illumination by means of a 15 watt bulb was maintained throughout the experiment.

According to Pringsheim (229), Detmer, as early as 1880, would have applied a flask, filled with water and equipped with an upward glass tube, in studying the water absorption in peas. Apparently, too little allowance has been made for the temperature, so that the apparatus acted chiefly as a thermometer.

No doubt the present method is liable to errors too. For instance, swelling takes place in the absence of air. It was presumed, however, that the gaseous exchange probably would play little part in the initial process of germination, viz. the water absorption, and that the intramolecular respiration would predominate. Moreover, the experiment took only seven to eight hours. All the methods, practised so far, are more or less inadequate and are lacking in accuracy to afford an insight into the subtle mechanism of the very first stage of germination.

The graphs in fig. 15 show the displacement of the menisci in the capillaries, brought about by various seeds. Although fairly large

fluctuations may be found between duplicate determinations, each kind of seed proved to yield a characteristic graph. Of course the size and the number of the seeds, thus the total amount of waterabsorbing material, will determine the ordinate value of the graphs.

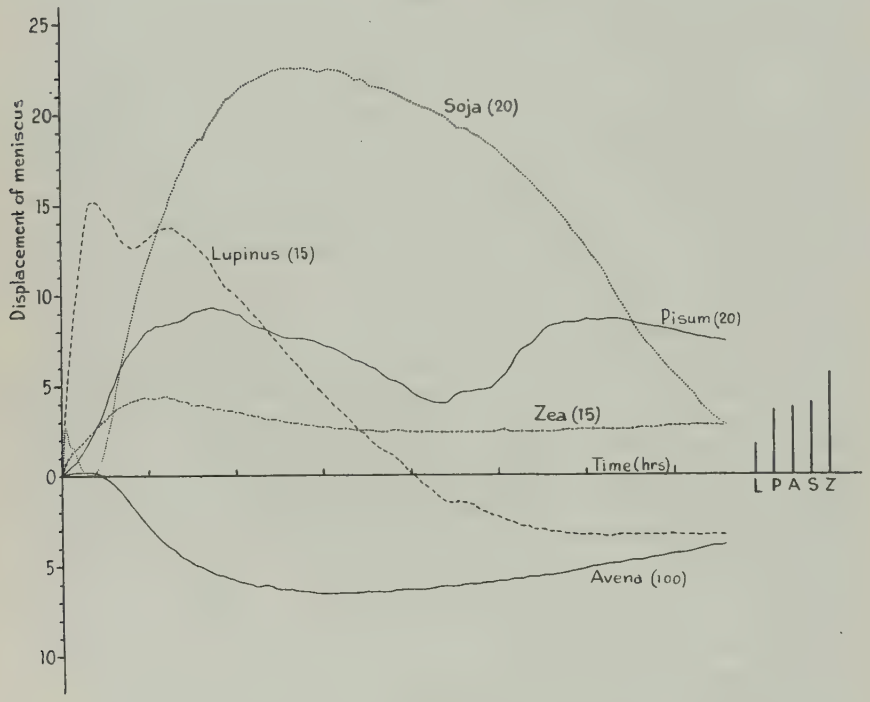


Fig. 15. "Swelling graphs" of five kinds of seeds; numbers in brackets. The displacement of the meniscus is rendered in centimetres along the ordinate; an increase in volume by a positive direction, and conversely. The verticals on the right represent a displacement corresponding with a change in volume by 1%, calculated on dry seed volume at the beginning of the test.

At first, it was tried to find some relation between the shape of the graphs and the observations both by PRAT (221–223) and by PRAT and CALVET (224–228), who studied the initial water absorption of seeds by means of a specially designed microcalorimeter. PRAT (223) arrived at the following conclusion: "immediately upon the contact between the dry seed and water there is a quick, then a falling off and then a more slowly developing production of heat. The initial phase of rapid rise and fall in the curve is common to both living and dead seed and is ascribed to *physico-chemical thermogenesis*. Then follows a phase of depression, often endothermic or weakly exothermic that was named "dead time" and finally the curve presents an increasing thermic flux ("*biological thermogenesis*") which corresponds to the beginning of the growth of the seedling, involving a progressive rising of its respiratory activity."

On closer examination of his own observations (checking the temperature during swelling; determining the rise of temperature required in obtaining a similar displacement by heat only) the present author was not able to ascertain any effect due to the evolution of heat. It is remarkable indeed that also these curves (fig. 15) were twin-peaked curves, whereas only the first part of a graph (as far as the pea is concerned, see below) could be reproduced after killing the seeds.

Some features of the water absorption in the pea are given here. Preliminary experiments with peas of one size soon revealed the presence of two different kinds in a given lot: *smooth* seeds and slightly *wrinkled* ones, behaving differently in soaking. There was much similarity in the amount of water absorbed, yet, the course of the process was different.

Smooth peas, in contact with water, developed local wrinklings on their seed-coat, spreading gradually over the surface until they covered the whole of the seed-coat. The final phase was marked by smoothing, the pea then being soaked with water.

Peas, somewhat *wrinkled* in the dry state, were seen to absorb the water more quickly at the whole of their surface. Here, absorption obviously took place with much more ease, so that nearly all the specimens had smoothly swollen when the test was finished. (The *smooth* peas, on the other hand, often contained specimens that would not swell beyond the wrinkled stage, or even such, remaining hard after the usual lapse of time).

The difference in behaviour between wrinkled peas and smooth ones also finds expression in the shape of the graph rendering the course of the water absorption; probably some diversity in the reserve substances must be held responsible for it. Wrinkled peas are lower in density than smooth ones, their water content is much the same.*)

In the water absorption curve of wrinkled peas the twin-peak is more pronounced than in that of smooth ones. The different slope clearly represents the quicker absorption in the former.

The descent following the first maximum corresponds to the gradual change from the wrinkled stage to that of complete swelling. Since, at any given moment, the seeds will not have swollen to the same degree, the results obviously will be variable.

Uniformity was favoured by using material of 6.0–6.5 mm diameter and of the same crop; as a rule the flasks contained twenty seeds.

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*) The divergencies observed in the swelling-tests between smooth peas and wrinkled ones was an incentive to study, as yet, the question whether the embryos from both types would develop differently.

Wrinkled peas, after pre-soaking, contain a higher percentage of specimens unfit for use than smooth seeds; many of them have a "marbled" appearance (perhaps owing to virus attack?). After a three weeks' culture the average lengths of the sprout and of the root plus hypocotyl in plants, grown from wrinkled seeds, were somewhat behind those obtained from smooth peas.

As, in the first part of this study, this fact was not taken into account a still more rigorous selection after sieving might contribute to lessen variability.

§ 3. Role of the seed-coat; water uptake by killed seeds

The shape of the water absorption curve, pictured in fig. 15, has raised a number of problems unsolved till now. Though, initially, water will be absorbed, the total volume was shown to increase; absorption is apparently attended by swelling. The decline of the curve probably corresponds to the penetration of water, after the seed-coat has been passed. Finally the peas, wrinkled by taking up water, changed into smoothly swollen ones; the increase in volume was distinctly visible and, judged from the curve, it surpassed the water uptake.

The resolution of these problems would require a separate investigation beyond the scope of the present work and, possibly, of a colloidochemical nature as well.

Some experiments on the role of the seed-coats in water absorption should not be omitted here. For that purpose, peas *without a seed-coat* were tested. Those with intact cotyledons produced already quite another curve (fig. 16, nr 2): after a slight decline a maximum appeared, the further course being nearly flat. Still less effect was produced when the cotyledons were isolated from the same number of peas (viz., twenty), so that forty loose organs were subjected to the swelling test (fig. 16, nr 3).

The water absorption by an adequate amount of small pieces of seed-coat was characterized by a curve being the inverse of nr 3 (fig. 16).

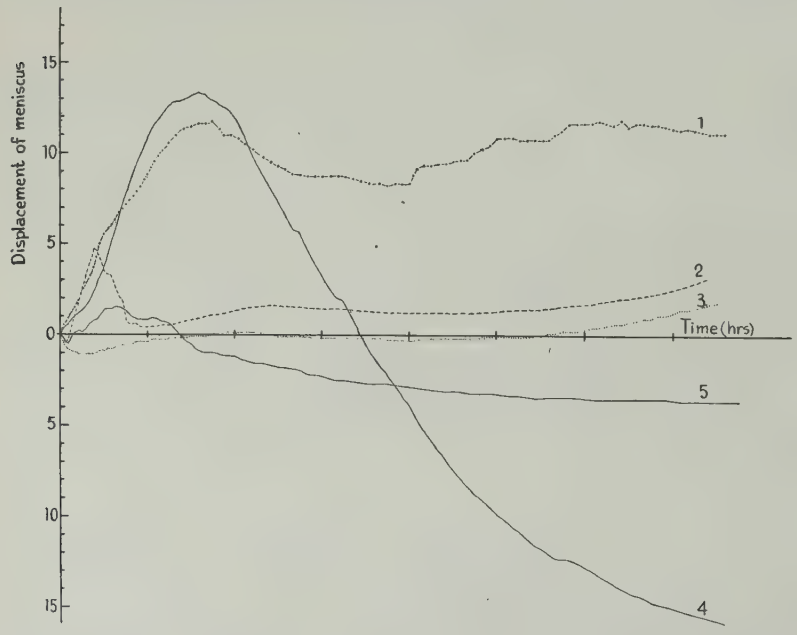

Fig. 16. Water uptake by smooth peas (20 specimens, 6.0 - 6.5 mm in diameter).
Graphs: 1. intact, normal seeds;
2. seeds without the seed-coats;
3. 40 loose cotyledons from similar peas;
4. intact peas, after killing (heating for 30 minutes at 100° C);
5. similar to 4, deprived from seed-coats after heating.

The circumstance that the curve changed completely when the seed-coat was removed proved the action of the whole seed to be different from that of the sum of its component parts. This suggested the presence of an ample amount of gases in the seed, which would determine the behaviour during water absorption. The above shows that, in the intact seed, the seed-coat may play an important part.

When it had been ascertained that dry peas, after heating at 100° C for half an hour, had lost their germinative power, this material was tested also (fig. 16, nr 4). The ascent of the curve, at first very similar to that of normal peas, was followed by a steady descent. So, there was a continuous absorption of water, whereas the total volume diminished. When the seed-coat was removed after killing the peas (fig. 16, nr 5) much of the effect was lost, just as with the viable seed.

§ 4. Excretion

In Chapter III (p. 26) there was mention of substances, given off by peas during pre-soaking in water. After the above swelling tests the soaking liquids were always evaporated to dryness on a steam bath. The residues, expressed in milligrams produced by 1 gram of air-dried starting material, were compared, and allowed of the following conclusions.

Wrinkled peas, in comparison with *smooth* ones, excrete nearly double the amount of matter (table XI). Killed seeds excrete *more* than viable ones, in both categories the wrinkled seeds again surpass the smooth ones. Finally, *maximal* excretion is found in *peas, deprived of their seed-coats*: twelve to seventeen times as much as in intact seeds, the wrinkled ones holding the record once more.

Excretion is enhanced by adding potassium hydroxide, whereas water saturated with carbon dioxide effects the reverse, the water absorption getting delayed.

From the observations that killed, intact peas excrete larger amounts of matter than viable ones, and even more in the absence of seed-coats (the distinction between viable and killed seeds having nearly vanished in that case) it might be inferred again that the seed-coat of *viable* seeds is important to the initial processes of germination.

§ 5. Influence of a growth substance

Both smooth and wrinkled peas were subjected to similar swelling tests in solutions of potassium indole-3-acetate, at concentrations of 10^{-4}, 3×10^{-4}, 10^{-3} and 5×10^{-3} mol. Except for the usual fluctuations, also found in the experiments with distilled water, no particular influence whatever of i.a.a.K was to be perceived. So, in seed treatment of the pea the growth substance probably will play little, if any, part in the initial phase of water absorption.

Summary

A new, sensitive method was applied in testing the initial water uptake in five kinds of seed. Each of them proved to yield a characteristic "swelling curve."

TABLE XI

Average values of water absorption, change in volume, and excretion, in *peas*, both smooth and wrinkled, under various conditions. Each test, lasting 7 to 8 hours, comprised 20 seeds, 6.0 - 6.5 mm in diameter.

Type of pea		Milieu	Weight (g)			Volume (ml)			Increase in volume, by displacement of meniscus in the capillary (mm^3)	Number of seeds swollen	Total amount of matter excreted (mg)
smooth	wrinkled		outset	end	increase	outset	end	increase			
	+	air	3.6	3.6	—	2.8	2.8	—	—	—	—
	+	water	3.6	7.0	3.4	2.8	6.2	3.4	48.0	19	38
+		air	3.8	3.8	—	2.9	2.9	—	—	—	—
+		water	3.8	6.7	2.9	2.9	5.9	3.0	75.9	16	20
+		water+CO_2	3.8	5.8	2.0	2.8	5.0	2.2	—	4	10
+		HCl 10^{-4} N	3.8	7.2	3.4	2.9	6.5	3.6	76.2	20	16
+		HCl 10^{-3} N	3.8	6.8	3.0	2.9	6.0	3.1	88.8	18	21
+		KOH 10^{-2}N	3.8	7.1	3.3	2.8	6.2	3.4	24.4	20	30

On the basis of more or less detailed observations, taken with the pea—in various states—it would seem that the first part of the graph is related to physico-chemical processes during water absorption, whereas the other part probably bears on physiological phenomena of the organism.

The seed-coat must be considered important, particularly to the excretion of certain substances by the seed. In view of the above tests, the question whether the initial absorption of water in peas would be influenced by i.a.a. during seed-treatment, has to be answered in the negative.

Chapter VII

SURVEY OF THE LITERATURE ON THE DISTRIBUTION OF NATURAL AUXIN IN SEEDS, DURING DEVELOPMENT, DORMANCY AND GERMINATION

The isolation of the auxins *a* and *b* from germinating *barley* and from *maize* seed oil, performed in 1934 by Kögl, Erxleben and Haagen-Smit (162) on the one hand, the discovery of the importance of the aleurone layer to seeds of various *Gramineae* by Schander (178) on the other underlie Cholodny's hypothesis (149). Immediately after taking up water, the endosperm would start forming a growth hormone ("Blastanin") as an enzymatic process, coupled with the hydrolytic degradation of amylum. Initial growth and subsequent development of the plant would then depend largely on the "hormone charge" received at the beginning of its life cycle.

Starting from this hypothesis, and on the assumption that the acceleration of development—to be observed after the vernalization of seeds—would result from an increasing amount of growth hormone in the meristematic parts of the embryo (150), Cholodny, in 1936 (25, 26), was the first to check whether normal plant growth might be influenced by administering a synthetic growth substance. This has induced the numerous investigations described in Chapter I.

On the basis of data from the literature it will be examined now as to what extent the above concept is supported by the findings of other workers and, merely on the score of theoretical considerations, how far any success is to be expected of treating seeds with synthetic growth substances. As to terminology, that from the original papers is retained as far as possible.

Experiments with the *Avena* coleoptile as a test-object raised a surmise that auxin would be present in the endosperm (171). In *maize*, the centre of growth hormone production would reside in the yellow, horny layer enclosing the endosperm, whereas in *Helianthus* the growth-stimulating substance is stored by the cotyledons (163). Cholodny's hypothesis, as such, cannot hold good for all cases, because

some growth hormone is already present in *dry* oat meal, while its formation starts soon after fertilization (163).

On the other side, DRABKIN (see under 233) demonstrated that the endosperm of vernalized seeds, as opposed to that of non-treated ones, fails to bring about any curvature in the Avena coleoptile. This suggests that the embryo absorbs the hormones of the endosperm in the initial developmental phase and provides additional evidence for CHOLODNY's hypothesis.

The use of non-specific test-methods (151, 170) has certainly given cause for a good deal of confusion and contradiction. POHL (172), after injuring the seed-coat and the aleurone layer, was able to remove the growth hormone from the endosperm by means of water or by applying a potential difference; eventually, he identified "Blastanin" with the growth hormone of the coleoptile. Thus, in his opinion heteroauxin and phenylacetic acid, which are unfit for stimulating the growth of coleoptiles from extracted seeds, should not be regarded as growth substances.

In *maize*, the active growth hormone of the endosperm would be converted into an inactive form during the passage through the scutellum. This substance might then be easily transported towards the top of the coleoptile, where re-activation to the auxin would take place. Some inhibitory substance (or growth substance antagonist) regulating the capability of the cells to react with the growth hormone, is very important to germination (172, 183, 184).

RUGE (176) completely shares the view of VON VEH and SÖDING (182), in that the growth hormone does not take part in the germination proper, that is, the change from dormancy to growth. The elongation-promoting hormone which does take part in primary development, therefore, should not be regarded as a *germination-hormone*.

AVERY, et al. (141, 142, 146), have compared various extraction methods when determining growth hormones in *maize*-endosperm during primary development. In dormant *maize*-endosperm, 90 per cent of the total auxin content exists as a physiologically inactive compound (in the Avena test), i.e. as a "precursor", which becomes auxin only after hydrolysis. Immediately after fertilization there is a rapid rise in auxin content; the maximum is attained in one to three weeks, after which a steady decrease sets in. There was *no relationship between vegetative vigour of hybrids and the amount of auxin stored in the kernels produced by them*, nor was there any relation between the auxin content of normal and polyploid forms (144). *Maize* seeds, the endosperm of which contains sugar, show a higher auxin content than those containing starch (145).

In *wheat* no relation was to be found between protein and auxin content. Probably two precursors are present, hydrolysis yielding two auxins distinguishable by their stability towards alkali (143).

In some respects the above meets the results obtained by HATCHER and GREGORY in their study of the auxin relations in *rye* varieties. The Avena test showed that, as soon as the presence of free auxin can be demonstrated (i.e., from three weeks after fertilization), here too the

auxin content increases during the next month, whereas it falls off during ripening. A maximum is found some five or six weeks after fertilization: the stage of complete differentiation of the embryo (160). In *rye*, auxin production would take place in the aleurone layer, close to the embryo (158; compare 151, 178).

According to HATCHER (159) the term "precursor" is misleading, since there is no evidence to date of its formation previous to the formation of auxin. It is quite possible that no specific, inactive substance is formed at all, but rather a protein-bound auxin. GORDON (153), after examining *wheat* grains, expresses himself in the same sense. During germination adequate amounts of growth hormone would then be set free in proteolysis.

Investigations by the school of GREGORY, by KONOVALOV and by SEN and CHAKRAVARTI (see under 237 and 238) demonstrated the possibility of vernalization of isolated embryos, independent of endosperm, aleurone layer or cotyledons. Thus, CHOLODNY's hypothesis in its original form has become untenable and it is even a question whether the hormone-metabolism is influenced by vernalization (159). It is worth mentioning that the loss of total auxin in ripening is relatively less in the earlier harvested ears. For that reason the highest auxin concentration is found in the most dwarfed grains (159).

A new era in the history of this plant hormone research was ushered in when, in 1941, HAAGEN-SMIT, LEECH and BERGREN (156) succeeded in isolating the growth hormone from fresh corn-meal (*Zea mais*) and, after identifying it with i.a.a., were the first to prove this to be a constituent of higher plants. From that time on it has been emphasized, anyhow, that what is usually called "auxin" may consist of more than one substance in the plant. It depends on the extraction-technique which of these is obtained (157).

One will have to take full account of the possibility that growth inhibitors may mask growth promotors, and reversely. The way in which this finds expression will depend on the test-method (152).

The free growth hormone of unripe *maize*-kernels, which attains its optimum concentration ten or fifteen days after fertilization, proves to be i.a.a. (155, 175). BERGER and AVERY (147, 148) make an attempt to elucidate the chemical character of the "precursor" in the resting *maize*-grain. Alkaline hydrolysis of this protein-bound substance yields i.a.a. as well.

VON GUTTENBERG and LEHLE-JOERGES (154) are also able to show that, besides auxin (*a* and *b*-type), several seeds contain an alkali-resistant substance, probably i.a.a. Extraction of swollen, germinating seeds shows an increase of the content after 24 hours, followed by a decrease after one or two days. *Leguminosae* (*Lupinus*, *Phaseolus*, *Pisum* and *Vicia*) give negative results.

From an investigation by MIROV (167) it would appear that resting seeds of *Pinus* do *not* contain any *active auxin*. This is only formed during stratification in cold storage; parallel with its appearance, the seeds are able to germinate. This speaks well once more for CHOLODNY's view and so does JUEL's paper (161), giving evidence of the relationship

between the decline of germination and the fall of the auxin content in seeds of *maize* and of *Phaseolus*.

In the germination of *Gramineae* root growth depends on the growth hormone content of the endosperm (169). The beneficial effect, or otherwise, of treating seeds with growth substances might be connected with this.

Some investigators imagine that the hormones in developing seed would act in the control of fruit drop rather than in further development (164).

In the more recent papers attention is paid not only to growth-promoting hormones but also to inhibitors, their mutual relations being considered as well (186–189). POHL and TEGETHOFF (173, 174, 181) isolated an inhibitory substance from *maize*-scutellum by electrodialysis. With the aid of MOEWUS's cress root test they examined the simultaneous action of inhibitor, active growth substance and inactive growth substance. The inhibitor of the endosperm, probably non-proteinaceous by nature, is able to inactivate both i.a.a. and the growth substance of the endosperm. The latter, in that case, gives rise to another substance, probably an inactive growth substance (181).

Here, the question arises whether, with the aid of this method, these separate actions can be specifically distinguished at all, considering the impossibility of doing so in determining the content of certain growth-promoting substances in *sunflower* seeds (176).

In connection with the problem of vernalization, SIRCAR and DAS (179) collected some data about the hormone content of *rice*; inhibitory substances would be absent. Likewise, LUCKWILL (165) did not find any relation between the germinative power of embryos isolated from *apple* seeds and their inhibitor content. The conclusion was that the formation of growth-producing substances rather than the disappearance of growth-inhibitors, may be necessary to break the dormancy of the seed.

MOEWUS and his co-workers (168), in reliance upon the results obtained with the well-known cress root test, are inclined to conclude that, in *Lepidium sativum*, two growth substances are present, viz. i.a.a. and, probably, phenylacetic acid. The i.a.a. content of fresh seeds, harvested in 1950, proved some 18 times as high as that of aged ones, of 1944 and 1947.

YAMAKI and NAKAMURA (185) applied the paper partition chromatography when determining tryptophane, indoleacetic acid and indoleacetaldehyde in germinating *maize*. Enzymes or enzyme systems which convert tryptophane, indole-3-ethylamine and indole-3-acetaldehyde into indole-3-acetic acid were found in the embryo of *Zea mais*. The genetic connections of tryptophane and i.a.a. in the embryo and endosperm were discussed, resting on the experimental results. The "bound" indole-3-acetic acid in the endosperm is considered as one of the decomposition products produced by hydrolysis with alkali and to be of very little importance from the physiological point of view.

DISCUSSION

From the literature cited in the foregoing chapter it is gathered that, generally, investigators agree that seeds contain growth-promoting substances, which are essential to development. There is no denying either that inhibitory substances play an important part. Perhaps one should concur in SÖDING's idea, viz. that the rest-period of the seed is generally caused by the presence of inhibitors. As they disappear, growth gradually starts, which requires growth hormones in an increasing measure. So, for the time being there is no sense in distinguishing special germination hormones.

Data about a diminishing content of the seed in aging and its relation to changes in germinative power are often contradictory. Thus, CHOLODNY's theory in its original form will not hold any longer.

The author, however, would like to stress that a serious mistake is made by trying to immediately generalize the results obtained on a single type. It should be properly realized that in this respect too, Nature is multifarious and that the interpretation of some data is accurate only under the set of conditions which prevailed during a given experiment.

As a whole, research on occurrence, isolation and identification of both growth-promoting and growth-inhibiting substances is far from complete; especially the *dicotyledons* have received very little attention on that score. Such experiments entail two main difficulties. First, during isolation (e.g., extraction) i.a.a. or some other growth substance may be formed owing to chemical action; second, in testing, the inhibitors present may mask the effect of growth-promotors, owing to the choice of the test-method (152). The application of modern techniques, e.g. chromatography (166, 185), certainly will conduce to new findings and to the elucidation of various, unexplained matters.

The administration of synthetic growth substances under certain circumstances will affect germination, growth and development, on the understanding that favourable results are obtained only incidentally (several instances are to be found in table II). This shows the details of the mechanism to be hidden still. The penetration of the growth substance administered, its transport, its accumulation in certain parts of the seedling and also further particulars will have to be studied with the aid of isotopic tracers. This is all the more desirable as it is still uncertain whether a beneficial effect of the synthetic growth substance is connected with an influencing of the hormone-metabolism of the seed. Other processes involved in germination may be influenced as well, favourably or otherwise: enzymatic processes in the developing embryos have been inadequately studied as yet. In this connection the recent investigation by MARRÈ and MURNEEK (242) is of consequence. They detected a considerable degree of resemblance between the natural growth hormones (unfolding their activity after fertilization) and the growth-regulators administered, as to their influencing the carbohydrate and hexose phosphate metabolism during the initial stages of seed formation.

The present author is fully aware of several shortcomings in this study. Among other things, the influence of light was eliminated in order to avoid complications. Therefore, the phenomena observed will not agree in every respect with those appearing under natural circumstances. A possible intervention of other substances, being of similar importance to the development of the young plant, was not considered either (233).

As to the penetration of a growth substance into the seed, any investigations whatsoever on the mode and the extent seem to be wanting. Though PRAT and CALVET (221–228) would have demonstrated that the "biological thermogenesis" is increased by indole-3-acetic acid at low concentrations, it follows from the present author's experiments on the initial water uptake in the pea that a growth substance (at least, i.a.a.) has little, if any, effect. So, its action probably will manifest itself mainly afterwards, i.e. after the pericarp, or the seed-coat or both of them have been passed. Interesting facts might come to light if other types of seed would be subjected to this test.

The distinction found between smooth peas and wrinkled ones is probably due to diversity in the reserve food. A varying ergon content may be connected herewith, which, in *maize*, was shown by AVERY, BERGER and SHALUCHA (145) and recently, by TEAS, CAMERON and NEWTON also (180). The divergent behaviour of varieties with regard to seed-treatment thus becomes more intelligible and future research will have to avail itself of carefully selected material in particular.

The present investigation has also shown that synthetic growth substances, administered to the seed, may unfold not only a direct action on the embryo, but also an indirect one, *via* the reserve substance mass. The existence of different types of seed — being distinguished by their reserve food — and the considerable variations in the ratio embryo-size/volume of reserve substances may be advanced as possible sources of the contradictory results obtained in seed-treatment.

One of the most outstanding responses of the embryo to growth substances, often shown, was a swelling of the hypocotyl attended with an increase of the number of lateral roots. Any appearing of larger yields on application in practice, *under certain circumstances* (condition of the soil, manuring, humidity, etc.: see p. 8) might be connected with this phenomenon.

For the sake of co-ordinating the activities of various workers it will be advisable to restrict future research to a well-grounded investigation on a few kinds of plants only. The desirability of commanding reliable methods by the aid of which the treatment of seeds with synthetic growth substances can be practised, also in the light of present-day endeavours to improve the world's food situation, certainly will justify such a fundamental investigation.

SUMMARY

In the treatment of seeds for the purpose of stimulating growth and development, the application of synthetic plant growth substances in

practice has not yet produced the desired result. This will be easily understood, considering the strongly divergent ways in which experiments have been made on a large number of different objects: the results, often contradictory, did not admit of any valuable conclusions.

A more profound knowledge of the process of germination and of the mechanism of growth substance action during the initial stages of development is deemed necessary if advance is to be made in this domain.

An investigation aiming at this purpose was carried out on the pea. Standardized, sterile conditions were maintained during the *in vitro* study of normal correlation-phenomena of the developing embryo and the influence exerted on it by various factors.

The effect of a growth substance (indole-3-acetic acid, naphthalene-1-acetic acid, 2,4-dichlorophenoxyacetic acid) can be clearly distinguished from that of other influences.

It is presumed that the obtaining of contradictory results in seed-treatment may be due chiefly to:
a) variability of the starting material; *b*) selecting action of the seed-coat, and *c*) the manner in which the growth substance is passed on to the young plant by the cotyledons or by the endosperm (filled with different reserve substances in different types of seed).

By means of various experiments it was ascertained that the growth substance administered acts upon the embryo not only directly, but also in an indirect way, like a thrust via the reserve substance mass. So, the role of the cotyledons would *not* be comparable to that of a reservoir gradually giving off the growth substance absorbed.

With the aid of a new, sensitive technique the initial water uptake was studied in five kinds of seed. The results could be recorded in characteristic swelling-curves. Although in this process and also in the exuding of certain substances the seed-coat plays an important part, no influence of indole-3-acetic acid on these processes in the pea could be ascertained.

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C. *Natural auxin distribution in seeds during development, rest-period and germination*

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REVISION EINIGER GATTUNGEN DER ASCOMYCETEN

VON

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In den letzten Jahren hat PETRAK in zahlreichen meist kleineren Mitteilungen eine grosse Anzahl neuer Gattungen und Arten der Ascomyceten und Fungi imperfecti beschrieben. Da die Diagnosen in einer unübersichtlichen, nur mit Mühe zu lesenden Form und meist ohne jegliche Illustration publiziert wurden, sind diese neuen Formen bisher grösstenteils unbeachtet geblieben oder wurden von andern Mykologen kritiklos hingenommen.

Bei meinen Studien über die amerosporen Pyrenomyceten und über den Verwandtschaftskreis der Gattung *Venturia* war ich gezwungen, auch die betreffenden von PETRAK beschriebenen Gattungen hinsichtlich ihrer systematischen Stellung näher zu untersuchen. Dabei musste ich feststellen, dass von diesem Autor zahlreiche Gattungen ohne die nötigen Vergleiche mit bereits bestehenden und ohne deren Verwandtschaft anzugeben aufgestellt wurden. Viele dieser Gattungen lassen sich denn auch nicht aufrecht erhalten. Einige davon sollen hier, andere in einer späteren Arbeit besprochen werden.

1. Atopospora Petrak

Ann. Mycol. *23*, 100 (1925)

Diese monotypische Gattung hat *Euryachora betulina* (Fr.) Schröter zur Typusart. PETRAK äusserte sich nicht über ihre systematische Stellung, sondern sagte nur, dass sie in ihrem innern Bau von der Typusart von *Euryachora* Fuck. vollständig verschieden sei und sich vor allem durch die Form und Farbe der Sporen unterscheide.

Euryachora betulina hat blattbewohnende, scharf begrenzte, schwarze, krustenförmig zwischen Kutikula und Epidermis wachsende Stromata. Diese enthalten zahlreiche einschichtig gelagerte Lokuli und sind senkrecht prosenchymatisch aufgebaut. Die dick- und derbwandigen Asci enthalten acht oft etwas ungleich zweizellige, erst hyaline, sich später braungrünlich färbende Sporen.

Uebereinstimmend gebaut ist die Gattung *Rehmiodothis* Theiss. et Syd. (1915). Die Autoren haben ihr zwar hyaline Sporen zugeschrieben; diese erhalten aber im Alter eine grünliche Farbe. *Atopospora* ist daher mit *Rehmiodothis* zu vereinigen und ihre Typusart hat **Rehmiodothis**

betulina (Fr.) comb. nov. (Syn.: *Dothidea betulina* Fries) zu heissen.

Rehmiodothis gehört in die Familie der *Venturiaceae* und ist mit *Stigmatea* Fr. und mit *Aphysa* Theiss. et Syd. am nächtsten verwandt. Bei den Vertretern dieser Familie färben sich die (oft ungleich) zweizelligen Sporen meist hell grünlich oder olivenbraun, seltener dunkel. Bei einigen Formen bleiben sie dauernd hyalin, aber allein auf der Sporenfarbe lassen sich innerhalb der *Venturiaceae* keine Gattungen basieren.

2. Metacoleroa Petrak
Ann. Mycol. *25*, 332 (1927)

Metacoleroa wurde für einen auf *Linnaea borealis* L. parasitierenden, am besten als *Venturia Dickiei* (Berk. et Br.) Sacc. bekannten Ascomyceten aufgestellt. Dieser bildet im Substrat subkutikulär und tiefer eine flache Stromakruste, aus der zahlreiche braune Hyphen hervorbrechen. Diese verzweigen sich mehrfach und bilden oberflächlich ein Subikulum, in dem die reichlich mit Bosten besetzten Fruchtkörper heranwachsen. Die zylinderisch-keuligen Asci besitzen eine dicke, doppelte Membran und enthalten acht zweizellige, grünliche oder olivenbraune Sporen.

Genau die gleiche Charakterisierung passt auch auf die Gattung *Dimerosporiopsis* P. Henn. (1901), nur sind dort die Perithecien nicht mit Borsten, sondern mit Hyphenhaaren besetzt. Die Typusart *D. Engleriana* P. Henn. et Nym. wächst in Südafrika auf Stämmchen von *Erica arborea* L. und wurde später erneut als *Aloysiella ruwenzovensis* Mattir. et Sacc. (1908) in eine eigene Gattung gestellt.

Wohl weil ihre Typusart auf einer *Ericaceae* wächst, hat Petrak (1947) die Gattung *Dimerosporiopsis* mit *Gibbera* Fries vereinigt. Seiner Ansicht nach sollen nämlich alle zu *Gibbera* gehörenden Arten auf Ericaceen wachsen. Dass sich Pilzgattungen nicht nach den Wirtspflanzen ihrer Vertreter charakterisieren lassen, liegt auf der Hand und braucht hier nicht näher diskutiert zu werden. Die Gattung *Gibbera* im Sinne von Petrak kann aber aufrecht erhalten bleiben, nur müssen dann die übereinstimmend gebauten, aber auf andern Wirtspflanzen wachsenden Pilze ebenfalls dazu gestellt werden.

Daher ist *Metacoleroa* als Synonym zu *Gibbera* und deren Typusart als *Gibbera Dickiei* (Berk. et Br.) v. Arx (1952) neben *Gibbera Engleriana* (P. Henn.) van der Bijl zu stellen.

Aus den gleichen Gründen müssen auch die Gattungen *Pseudotthia* P. Henn. (1899), *Dothidotthia* v. Höhn. (1919) und *Xenomeris* Syd. (1924) mit *Gibbera* vereinigt werden. Bei den Arten dieser Gattungen sind zwar die Gehäuse kahl; oft finden sich aber oberflächliche, dematoide Hyphen oder Reste einer dazu gehörenden, hyphomycetenartigen Konidienform (vgl. Müller und v. Arx, 1950; v. Arx, 1952).

Die bisher bei den zuletzt erwähnten Gattungen untergebrachten, sowie andere zu *Gibbera* zu stellende Arten sollen kurz aufgezählt werden:

1. **Gibbera pseudotthia** Müller et v. Arx — Ber. Schweiz. Bot. Ges. *60*, 368 (1950)

Syn.: *Pseudotthia vaccinii* P. Henn. et Nym.—Monsunia *1*, 69 (1899); Matr.: Auf dürren Blättern von *Vaccinium varingaefolium* Mig. (Java). Diagn.: v. Höhnel (1909).

2. **Gibbera symphoricarpi** (Rehm) comb. nov.

Syn.: *Pseudotthia symphoricarpi* Rehm—Ann. Mycol. *11*, 169 (1913); *Dothidotthta symphoricarpi* v. Höhn.—Sitzber. K. Akad. Wiss. Wien, math.-nat. Kl., I, *128*, 593 (1919); *Dibotryon symphoricarpi* Petr.—Ann. Mycol. *25*, 301 (1927); Matr.: Auf Aestchen von *Symphoricarpus occidentalis* Hook (Nordamerika). Diagn.: v. Höhnel (l.c.)

3. **Gibbera spiraeae** (Murash.) comb. nov.

Syn.: *Systremma spiraeae* Murash. cit. Petrak–Ann. Mycol. *25*, 300 (1927); *Dibotryon spiraeae* Petr.—Ann. Mycol *25*, 301 (1927). Diagn.: Petrak (1927) gibt wohl eine sehr umfangreiche Diagnose, zitiert aber die Originalbeschreibung nicht und nennt auch keine Nährpflanze.

4. **Gibbera pruni** (Syd.) comb. nov.

Syn.: *Xenomeris pruni* Syd.—Ann. Mycol. *22*, 185 (1924). Matr.: Auf Blättern von *Prunus lusitanica* L. (Kanarische Inseln). Diagn.: Sydow (l.c.).

5. **Gibbera eucalypti** (Syd.) comb. nov.

Syn.: *Xenomeris eucalypti* Syd.—Ann. Mycol. *28*, 73 (1930). Matr.: Auf Blättern von *Eucalyptus* und anderen Pflanzen (Südamerika). Diagn.: Sydow (l.c.).

6. **Gibbera alpina** (Petr.) comb. nov.

Syn.: *Xenomeris alpina* Petr.—Sydowia *1*, 101 (1947). Matr.: Auf dürren Blättern von *Vaccinium vitis idaea* L. (Alpengebiet). Diagn.: Petrak (l.c.).

7. **Gibbera examinans** (Berk. et Curt.) comb. nov.

Syn.: *Asterina examinans* Berk. et Curt.—Fungi Cubensis no. 737 (1869); *Montagnina examinans* v. Höhn.—Sitzber. K. Akad. Wiss. Wien math.-nat. Kl., I, *119* (No. 488) (1910). Matr.: Auf Blättern eines unbekannten Baumes (Kuba). Diagn.: v. Höhnel (l.c.). Dieser Autor hat für diese mit *Gibbera vaccinii* Fr. verwandete Art eine eigene Gattung *Montagnina* aufgestellt die ebenfalls als Synonym zu *Gibbera* gestellt werden muss.

8. **Gibbera Ramakrishnani** nom. nov.

Syn.: *Achorella vaccinii* T. S. Ramakrishnan—Proc. Ind. Acad. Sci. *34*, 64 (1952). Matr.: *Vaccinium leschnaultii* Wight (Indien). Diagn.: Ramakrishnan (l.c.).

Diese Art besitzt ein krustenförmiges Stroma, dem die kahlen Gehäuse traubig aufsitzen. Die 90–130 × 11–13 μ grossen Asci enthalten acht etwas ungleich zweizellige, sich bei der Reife olivenbraun färbende, 11–15 × 5–7 μ grosse Sporen.

Gibbera symphoricarpi und *G. spiraeae* wurden von Petrak (1927) zu *Dibotryon* Theiss. et Syd. gestellt. Diese Gattung gehört ebenfalls zu den *Venturiaceae* und steht *Gibbera* sehr nahe, unterscheidet sich aber durch die lange hyalin bleibenden, birnförmigen, nahe dem untern Ende septierten, also apiosporen Sporen und durch die grossen, oft mehrere Zentimeter langen, krebsartige Geschwüre verursachenden Stromata, in denen sich vorerst eine *Hormodendron*-artige Konidienform bildet, ferner durch die verhältnismässig grossen Gehäuse. Die

Typusart *Dibotryon morbosum* (Schw.) Theiss. et Syd. wurde von Koch (1935) beschrieben und gut abgebildet. Sie parasitiert in Nordamerika auf Zweigen verschiedener *Prunusarten* und hat 14–21 × 5–8 μ grosse Sporen; die untere Zelle misst 3.5–5 × 2–3 μ.

Leider lässt sich die Gattung *Dibotryon* Theiss. et Syd. (1915) nicht aufrecht erhalten und muss mit *Apiosporina* v. Höhn. (1910) vereinigt werden. Deren Typusart *A. collinsii* (Schw.) v. Höhn. = *Sphaeria collinsii* Schw. stimmt im Baue der *Hormodendron*-artigen Konidienform und der Ascusform vollkommen mit *Sphaeria morbosa* Schw. überein, nur handelt es sich bei ihr um einen Blattparasiten auf *Amelanchier*arten (Sartoris und Kauffman, 1925). Sie hat länglich-keulige oder birnförmige, erst hyaline, sich später grünlich-braun färbende, nahe dem untern Ende septierte, 11-16 × 3.5-5 **g** grosse Sporen.

Sphaeria morbosa Schw. = *Plowrightia morbosa* Sacc. = *Dibotryon morbosum* Theiss. et Syd. muss daher als **Apiosporina morbosa** (Schw.) comb. nov. eingereiht werden.

Die Gattung *Botryostroma* v. Höhn. (1911) hat mit *Apiosporina* die sehr ungleich zweizelligen, erst hyalinen, später gebräunten Sporen gemeinsam und ist damit nahe verwandt, lässt sich aber durch die Wachstumsweise und die fehlenden Konidien unterscheiden. *Botryostroma inaequale* (Wint.) v. Höhn. wächst ohne Fleckenbildung auf lebenden Blättern, auf denen die kleinen, kahlen Gehäuse in dichten Herden von 1–3 mm Grösse einem subkutikulären oder intraepidermalen, oft unterbrochenen Hypostroma aufsitzen.

Die Gattung *Rosenscheldiella* Theiss. et Syd. (1915) wiederum stimmt mit *Botryostroma* in ihrer Wachstumsweise und im Baue der Fruchtschicht vollkommen überein, nur sind dort die sehr lange hyalin bleibenden Sporen in der Mitte septiert und bei der Querwand nicht eingeschnürt. Auch diese Gattung gehört zu den *Venturiaceae* und ist mit *Gibbera* nächst verwandt, muss aber davon auf Grund derselben Merkmale getrennt werden, durch die *Botryostroma* von *Apiosporina* unterschieden werden kann.

Die Gattung *Apiodothina* Pet. et Cif. (1932) dagegen ist möglicherweise von *Botryostroma* nicht verschieden und wäre damit zu vergleichen. Jedenfalls gehört auch sie zu den *Venturiaceae*.

3. Neogibbera Petrak
Sydowia *1*, 191 (1947)

Diese Gattung soll sich von *Gibbera* durch fast opak schwarzbraune Sporen, sowie durch die dunkel blauschwarz gefärbten, mit subhyalinen Verdickungsleisten versehenen Gehäusezellen und durch die borstenartigen, dem Hypostroma aufsitzenden Hyphen unterscheiden. Die vier beschriebenen Arten parasitieren auf Blättern tropischer Fagaceen. Mit andern Ascomycetengattungen wurde *Neogibbera* vom Autor nicht verglichen.

Wie schon aus der Diagnose hervorgeht, fällt *Neogibbera* mit *Acantharia* Theiss. et Syd. (1918) zusammen. *A. echinata* (Ellis et Ev.)Theiss. et Syd. = *Dimerosporium echinatum* Ellis et Ev. als bisher einzige Art der

Gattung wächst ebenfalls auf einer tropischen *Fagaceae*, nämlich auf Blättern von *Quercus chrysolepis* Liebm. in Kalifornien. Die Diagnosen der beiden Gattungen stimmen in allen generisch wichtigen Merkmalen völlig überein (vgl. auch HANSFORD, 1946). Die Arten der Gattung *Neogibbera* müssen daher zu *Acantheria* gestellt werden, woraus sich folgende Neukombinationen ergeben:

1. **Acantharia hamata** (Penz. et Sacc.) comb. nov.

Syn.: *Dimerosporium hamatum* Penz. et Sacc.—Malpighia *11*, 389 (1897); *Neogibbera hamata* Petr.—Sydowia *1*, 191 (1947). Matr.: Auf lebenden Blättern von *Quercus* spec. (Java). Diagn.: PETRAK (l.c. p. 183 Seitenmitte).

2. **Acantharia aterrima** (Cke. et Wint.) comb. nov.

Syn.: *Dimerosporium aterrimum* Cke. et Wint.—Grevillea *20*, 83 (1892); *Neogibbera aterrima* Petr.—Sydowia *1*, 191 (1947). Matr.: Auf lebenden Blättern einer *Quercus*- oder *Pasania*-Art (Indien). Diagn.: PETRAK (l.c. p. 182).

3. **Acantharia elegans** (Syd.) comb. nov.

Syn.: *Dimerium elegans* Syd.—Ann. Mycol. *7*, 174 (1909); *Neogibbera elegans* Petr.—Sydowia *1*, 191 (1947). Matr.: Auf lebenden Blättern von *Pasania cuspidata* Oerst. (Japan) Diagn.: PETRAK (l.c. p. 186).

4. **Acantharia sinensis** (Petr.) comb. nov.

Syn.: *Neogibbera sinensis* Petr.—Sydowia *1*, 192 (1947). Matr.: Auf lebenden Blättern von *Quercus semicarpifolia* Sm. (China). Diagn.: PETRAK (l.c. p. 185).

4. Punctillum Petr. et Syd.
Ann. Mycol. *22*, 368 (1924)

Diese Gattung wurde für einen in Neuseeland auf Blättern eines Lebermooses gesammelten und als *Laestadia hepaticarum* Cke. beschriebenen Pilz aufgestellt. Wie aus der Diagnose hervorgeht, handelt es sich um eine kleine *Venturiaceae* mit halbkugeligen, kleinen, unten hellen, oben etwas dunkelwandigen, kahlen Gehäusen.

Die Gattung *Punctillum* lässt sich neben *Stigmatea* Fr. nicht aufrecht erhalten und ihre Typusart muss als **Stigmatea hepaticarum** (Cke.) comb. nov. eingereiht werden.

5. Pseudodimerium Petrak
Ann. Mycol. *22*, 21 (1924)

Diese monotypische Gattung wurde bei ihrer Aufstellung nur mit *Dimerosporium* Fuck. verglichen. Ihre Typusart *P. meliolicolum* Petr. parasitiert auf *Meliola nidulans* (Schw.) Cke., besitzt ein oberflächliches, braunes Mycel ohne Hyphopodien und völlig oberflächlich dem Mycel aufsitzende, kleine, rundliche, erst geschlossene, kahle Gehäuse. Die dickwandigen Asci enthalten acht zweizellige, gefärbte Sporen.

Für Hyperparasiten mit den angegebenen morphologischen Eigenschaften besteht die Gattung *Dimerium* Sacc. et Syd. (1905), mit der *Pseudodimerium* daher zusammenfällt. Ihre Typusart hat bereits HANSFORD (1946) als *Dimerium meliolicola* (Petr.) Hansf. eingereiht.

6. Neodimerium Petrak
Sydowia *4*, 341 (1950)

Diese Gattung soll sich vor allem durch ein oberflächliches, mit aufsteigenden Seitenästen versehenes, braunes Mycel auszeichnen. Diesem sitzen die rundlichen, kahlen, dunkel- und ziemlich dickwandigen Gehäuse auf. Die wenig zahlreichen Asci enthalten acht zweizellige, im Alter gefärbte Sporen.

Petrak vergleicht diese Gattung mit der im Wirklichkeit ganz anders gebauten *Balladynopsis* Theiss. et Syd. und kommt zum Schluss, dass sein Pilz nicht dazu gehören könne und daher zu den *Dimerieen* gestellt werden müsse. Von allen bisher bekannt gewordenen Gattungen dieser Gruppe würde er sich durch das dem Substrate nicht fest anliegende und mit Borsten versehene Mycel unterscheiden.

Neodimerium ist ohne Zweifel eine *Parodiopsideae* und muss mit der Gattung *Parodiopsis* Maubl. (1915) sensu Arnaud (1921) vereinigt werden. **Parodiopsis Sydowii** (Petr.) comb. nov. (syn.: *Neodimerium Sydowii* Petr.) ist eine kleinere Art mit verhältnismässig lockerem Mycel. In ihrem Bau stimmt sie weitgehend mit *Parodiopsis splendens* (Pat.) Arn. überein, ist aber in allen Teilen kleiner. Für die letztgenannte Art hatte Theissen (1916) eine eigene Gattung *Piline* aufgestellt, die ebenfalls mit *Parodiopsis* zusammenfällt.

7. Episphaerella Petrak
Ann. Mycol. *22*, 126 (1924)

Die Typusart dieser für *Dimerosporium manihotis* P. Henn. begründeten Gattung hatte Arnaud (1921) als *Parodiopsis* eingereiht. Petrak hat *Episphaerella* als eine oberflächlich wachsende *Mycosphaerella* mit kahlen, unten durch hyaline oder braune, meist einfache Hyphen locker aufgewachsenen Gehäusen charakterisiert.

Genau den gleichen Bau haben die von Hansford (1946) in die Gattung *Eudimeriolum* Speg. (1912) gestellten Pilze. Die kahlen, kugeligen, oberflächlich wachsenden Gehäuse von *E. gynosporiae* Hansf. zum Beispiel sind unten mit spärlichen, braunen Hyphen besetzt, welche durch die Stomata ins Blattinnere dringen, sich in den Atemhöhlen verzweigen und oft parenchymatische Komplexe hyaliner Zellen bilden.

Bei der Diagnose von *Episphaerella manihotis* äussert sich Petrak mit keinem Wort über intramatrikale Hyphen oder Zellkomplexe. Wie aber aus der von Arnaud (1921) gegebenen Abbildung hervorgeht, sind solche auch bei dieser Art reichlich vorhanden und durchwuchern oft grosse Teile des Mesophylls.

Die Gattung *Episphaerella* muss daher mit *Eudimeriolum* vereinigt werden und ihre Typusart ist als **Eudimeriolum manihotis** (P. Henn.) comb. nov. einzureihen. *Eudimeriolum* ist wohl mit *Parodiopsis* verwandt, unterscheidet sich aber durch kleinere Gehäuse, nur spärliche, oberflächliche, borstenlose Hyphen und durch die scheinbar bleibend hyalinen Sporen. Die Gattung wird am besten bei den *Dimerieen* untergebracht.

8. Xenostigmella Petrak

Sydowia *4*, 369 (1950)

Diese Gattung wurde von Petrak bei ihrer Aufstellung nur mit *Xenostigme* Syd. verglichen, von dem sie sich durch den pseudoparenchymatischen, nicht prosenchymatischen Bau der sich am Scheitel mit einem Porus öffnenden, nicht schleimig zerfallenden Membran (= Gehäusewand!) und durch die charakteristischen Hyphopodien unterscheiden soll.

Wahrscheinlich ist *Xenostigmella* mit *Xenostigme* nicht näher verwandt, stimmt aber in seinem innern und äussern Bau vollkommen mit *Balladynopsis* Theiss. et Syd. (1917) überein und fällt damit zusammen.

Kaum ein Jahr nach der Aufstellung von *Xenostigmella* hat Petrak (1951) die Gattung *Balladynopsis* neu charakterisiert und *Wageria* Stevens et Dalbey wie auch *Balladynastrum* Hansf. damit vereinigt. Dagegen erwähnt er hier *Xenostigmella* mit keinem Wort. Möglicherweise ist dessen Typusart *X. paradoxa* Petr. mit einer andern Art der Gattung *Balladynopsis* identisch, was sich aber erst nach dem Vergleich der betreffenden Exemplare feststellen lässt. Vorläufig muss die Art als **Balladynopsis paradoxa** (Petr.) comb. nov. eingereiht werden.

9. Xerodiscus Petrak

Denkschr. Ak. Wiss. Wien, math.-nat. Kl. *105*, 2, 16, (1943)

Diese nur kurz beschriebene Gattung soll eine Zwischenstellung zwischen den Myriangiaceen und den echten Discomyceten einnehmen. Von ihrer Typusart konnten ein Originalexemplar, sowie mehrere als *Arthonia dispersa* (Schrad.) Rehm bestimmte Kollektionen untersucht werden. *Xerodiscus Rechingeri* Petr. ist in der Tat mit *Arthonia dispersa* identisch. Bei der als *Xerodiscus* bestimmten Kollektion sind die Ascosporen zweizellig, bei einigen andern fand ich sie vierzellig. Prächtig entwickelt war der Pilz in einer von Frl. H. F. J. van der Brugge in Spanien auf *Rosmarinus officinalis* L. gesammelten Kollektion. Hier fand ich in ein und demselben Fruchtkörper 12–16 × 5–7.5 μ grosse Sporen mit einer oder mit drei Querwänden.

Lecidiopsis cembrina (Anzi) Rehm und *Arthonia cytisi* Mass. sind ebenfalls Synonyme dieser vor allem im Mittelmeergebiet sehr häufigen Art.

Die Gattung *Xerodiscus* Petr. fällt mit *Arthonia* Achar. (1806) im Sinne von Rehm (1896) völlig zusammen. *Lecidiopsis* (Almq.) Rehm soll sich durch zweizellige Sporen unterscheiden und lässt sich neben *Arthonia* ebenfalls nicht aufrecht erhalten.

Die *Arthoniaceae* sind eine ziemlich isoliert stehende, zu den *Dothiorales* sensu Müller und v. Arx (1950) zu stellende Familie. Zahlreiche, aber nicht alle Vertreter leben in Symbiose mit Algenzellen oder sind Flechtenparasiten. Es scheint, dass Gonidien bei ein und derselben Art vorhanden sein oder fehlen können. Man könnte geneigt sein, die wie *Arthonia* gebauten Pilze ohne Gonidien in eine besondere

Gattung zu stellen. Nach ZAHLBRUCKNER (1926) entsprechen auch der Flechtengattung *Arthonia* zahlreiche Pilzgattungen wie *Celidiopsis* Massal., *Celidium* (Tul.) Koerb., *Conida* Massal., *Lecideopsis* (Almq.) Rehm und *Mycarthonia* Reinke. (vgl. auch NANNFELDT, 1932). Derartige Gattungen wurden dann auch von REDINGER (1937) und von SANTASSON (1952) wieder mit *Arthonia* vereinigt.

Sehr nahe mit *Arthonia* verwandt ist auch die Gattung *Protoscypha* Syd. (1925). Bei der Typusart *Protoscypha subtropica* (Wint.) Syd. bilden die Sporen aber meist ausser den Querwänden eine unvollständige Längswand, ferner unterscheidet sich der Pilz auch biologisch durch seinen Parasitismus auf Arten der Pyrenomycetengattung *Coccostroma* Theiss. et Syd. Die Gattung *Protoscypha* kann aufrecht erhalten bleiben, muss aber bei den *Arthoniaceae* eingereiht werden.

Dagegen müssen nach SANTESSON (1952) die beiden Gattungen *Manilaea* Syd. (1914) und *Eremotheciella* Syd. (1917) mit *Arthonia* vereinigt werden.

10. Cucurbidothis Petrak
Ann. Mycol. *19*, 201 (1921)

Diese für *Cucurbitaria pithyophila* (Fr.) de Not. aufgestellte Gattung soll nach PETRAK echt dothideal gebaut sein, während die echten *Cucurbitaria*-Arten zu den *Sphaeriales* gehören sollen. In Wirklichkeit ist auch *Cucurbitaria* Gray ein Vertreter der *Pseudosphaeriales* (*Dothideales* sensu PETRAK) und ist am nächsten mit *Teichospora* Fuck. verwandt. Die Gattung zeichnet sich durch verhältnismässig grosse, rasig einem Hypostroma aufgewachsene Fruchtkörper und durch braune, mauerformig geteilte, einreihig im Ascus liegende Sporen aus (MUNK, 1953).

Die Gattung *Cucurbidothis* ist tatsächlich mit *Cucurbitaria* nicht näher verwandt, lässt sich aber neben *Gibberidea* Fuck. (1869) nicht aufrecht erhalten. Bei *Gibberidea visci* Fuck. als Typusart haben die Sporen wohl mehrere Querwände, aber keine Längswand. Diese wird aber in den sonst gleich gebauten Sporen von **Gibberidea pithyophila** (Fr.) comb. nov. (Syn.: *Sphaeria pithyophila* Fr., *Cucurbidothis pithyophila* Petr.) ebenfalls nur selten ausgebildet. In einer von mir nachgeprüften, sicher gut ausgereiften Kollektion konnte ich überhaupt keine Sporen mit Längswänden finden.

Bei **Gibberidea conjuncta** (Petr.) comb. nov. (Syn.: *Cucurbidothis conjuncta* Petr. — Ann. Mycol. *20*, 188, 1922 — auf Aesten von *Thuja plicata* Don in Nordamerika) scheinen die Sporen ebenfalls keine Längswand auszubilden.

Die Gattungen *Rosenscheldia* Speg. (1883) und *Naumovia* Dobr. (1928) stimmen in ihrem Bau völlig mit *Gibberidea* überein und wurden bereits von SHEAR (1937) damit vereinigt. Dagegen hat PETRAK (1941) an der Gattung *Rosenscheldia* festgehalten und für *Gibberidea abundans* (Dobr.) Shear die unnötige Kombination *Rosenscheldia abundans* (Dobr.) Petr. gebildet.

PETRAK (1941) glaubt, *Rosenscheldia* (= *Gibberidea*) sei von *Leptosphaeria* nur durch die oberflächlich einem Hypostroma aufsitzenden

und nicht dem Substrate eingesenkten Fruchtkörper verschieden. Ob diese Ansicht richtig ist, müsste noch näher geprüft werden. Ich möchte aber darauf aufmerksam machen, dass sich bei allen echten *Leptosphaeria*-Arten in Sinne von MÜLLER (1950) die Früchtkörper in der meist papillenförmig vorgezogenen Scheitelmitte mit einem engen, rundlichen Porus oder Kanal öffnen. Bei *Gibberidea* dagegen sind die Gehäuse oben mehr oder weniger flach und öffnen sich durch Wegbröckeln grösserer Scheitelpartien mit einem weiten Loch. Sie stimmen hierin mit den Vertretern der *Dothioraceae* oder *Botryosphaeriaceae* überein. Auch nach dem Baue der Fruchtschicht, der Asci und Sporen beurteilt ist *Gibberidea* ein typischer Vertreter der *Dothiorales*.

11. Arnaudiella Petrak
Ann. Mycol. *25*, 339 (1927)

In der Sylloge fungorum von SACCARDO ist die Gattung *Seynesia* Sacc. als *Microthyriaceae* mit oberflächlich dem Substrate aufsitzenden, schildförmigen, radiär gebauten Fruchtkörpern und zweizelligen, gefärbten Ascosporen charakterisiert. PETRAK (1927) hat nun die Typusart *Seynesia nobilis* (Welw. et Curr.) Sacc. untersucht und gefunden, dass diese mit *Pemphidium erumpens* (Berk. et Curt.) Sacc. identisch ist. Dieser Pilz, den er dann *Seynesia erumpens* (B. et C.) Petr. genannt hat, gehört zu den *Sphaeriales* und zeichnet sich durch die dem Substrate eingesenkten und von einem Klypeus bedeckten, flach linsenförmigen Perithecien aus.

Ich konnte nun *Pemphidium erumpens* ebenfalls untersuchen und habe gefunden, dass diese Art mit *Pemphidium nitidum* Mont., der Typusart der Gattung *Pemphidium* Mont. in ihrem Bau völlig übereinstimmt. Nur ist der Pilz in der Originalkollektion von *P. nitidum* noch unreif und schlecht entwickelt, weshalb die Ascosporen in der Literatur auch als einzellig angegeben wurden. Jedenfalls fällt die Gattung *Seynesia* Sacc. (1883) emend. PETRAK (1927) mit *Pemphidium* Mont. (1840) zusammen. Darüber soll an anderer Stelle ausführlich berichtet werden.

PETRAK (1927) hat nun für die wie *Microthyrium* gebauten und nur durch gefärbte Sporen abweichenden, bisher fälschlicherweise bei *Seynesia* untergebrachten Arten rein theoretisch die Gattung *Arnaudiella* aufgestellt. Als Typusart bezeichnet er *Seynesia caronae* Pass., scheint aber diesen Pilz nicht untersucht zu haben, wenigstens gibt er von ihm keine Diagnose.

Für Formen ohne freies Mycel, aber mit oberflächlich dem Substrate aufsitzenden, schildförmigen, radiär gebauten Fruchtkörpern und zweizelligen, gefärbten Sporen bestehen aber die älteren Gattungen *Seynesiella* Arnaud (1918), *Hariotula* Arn. (1918) und *Seynesiola* Speg. (1919). Von diesen erwähnt PETRAK (1927) nur *Seynesiella* und behauptet, sie sei von ARNAUD für mycellose *Asterina*-Arten aufgestellt worden. Dies entspricht aber keinesfalls den Tatsachen. Für Formen ohne oberflächliches Mycel, aber mit *Asterina* (*Asterinella*) Fruchtkörpern hat ARNAUD die Gattung *Hariotula* begründet. *Seynesiella* da-

gegen charakterisiert er als *Microthyrium* mit gefärbten, zweizelligen Sporen. Rein theoretisch beurteilt fällt daher die theoretisch aufgestellte Gattung *Arnaudiella* Petr. mit *Seynesiella* Arn. zusammen. Ob dies wirklich der Fall ist, kann nur durch Vergleich der betreffenden Originalexemplare festgestellt werden. Ich konnte aber vorläufig nur *Seynesiella juniperi* (Desm.) Arn. untersuchen. Dieser Pilz ist mit *Stigmatea* nahe verwandt und unterscheidet sich nur durch sein oberflächliches, nicht subkutikuläres Wachstum. Es handelt sich hier ohne Zweifel um eine durch das Substrat beeinflusste, an das Wachstum auf *Juniperus*-Nadeln angeglichene, sonst typische *Venturiaceae*.

12. Phragmodimerium Petr. et Cif.
Ann. Mycol. *30*, 230 (1932)

Phragmodimerium insigne Petr. et Cif. als Typusart dieser monotypischen Gattung parasitiert auf dem Stroma einer *Hypocrella*, besitzt ein oberflächliches, braunes Mycel ohne Hyphopodien und Borsten, oberflächlich dem Mycel aufsitzende, rundlich-eiförmige, erst geschlossene Fruchtkörper, dickwandige Asci und schwarzbraune, mit drei Querwänden versehene Ascosporen.

Wie aus diesen kurzen Angaben hervorgeht, stimmt *Phragmodimerium* in allen generisch wichtigen Merkmalen mit *Philonectria* Hara (1914) überein (vgl. Hansford, 1946). *P. variabilis* Hara als Typusart parasitiert auf einer *Nectria* in Japan. *Phragmodimerium* ist deshalb mit *Philonectria* zu vereinigen und *Phragmodimerium insigne* Petr. et Cif. muss **Philonectria insigne** (Petr. et Cif.) comb. nov. genannt werden.

Summary

This paper gives a revision of some genera of the *Ascomycetes*, described as new by Petrak and other authors. The following genera are synonymous and must be placed in the earlier described genera:

Aloysiella Mattir. et Sacc. (1908) = *Gibbera* Fr. (1849)
Atopospora Petr. (1925) = *Rehmiodothis* Theiss. et Syd. (1915)
Arnaudiella Petr. (1927) = *Seynesiella* Arn. (1918)
Cucurbidothis Petr. (1921) = *Gibberidea* Fuck. (1869)
Dibotryon Theiss. et Syd. (1915) = *Apiosporina* v. Höhn. (1910)
Dothidotthia v. Höhn. (1919) = *Gibbera* Fr. (1849)
Episphaerella Petr. (1924) = *Eudimeriolum* Speg. (1912)
Lecideopsis (Almq.) Rehm (1896) = *Arthonia* Achar. (1806)
Metacoleroa Petr. (1927) = *Gibbera* Fr. (1849)
Montagnina v. Höhn. (1910) = *Gibbera* Fr. (1849)
Neodimerium Petr. (1950) = *Parodiopsis* Maubl. (1915)
Neogibbera Petr. (1947) = *Acantharia* Theiss. et Syd. (1918)
Phragmodimerium Petr. et Cif. (1932) = *Philonectria* Hara (1914)
Pseudodimerium Petr. (1924) = *Dimerium* Sacc. et Syd. (1905)
Pseudotthia P. Henn. (1899) = *Gibbera* Fr. (1849)
Punctillum Petr. et Syd. (1924) = *Stigmatea* Fr. (1849)

Seynesia Sacc. emend. Petr. (1927) = *Pemphidium* Mont. (obligate)
Xenomeris Syd. (1924) = *Gibbera* Fr. (1849)
Xenostigmella Petr. (1950) = *Balladynopsis* Theiss. et Syd. (1917)
Xerodiscus Petr. (1943) = *Arthonia* Achar. (1806)

As far as necessary, the type species of these genera and some other species have been renamed.

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NOTES ON AMERICAN MUSCI

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Papillaria nigrescens (Hedw.) Jaeg. and **P. appressa** (Hornsch.) Jaeg.

The variability of *Papillaria nigrescens* has already been emphasized by Steere (1934) and Bartram (1949). Even in the type specimen (S-PA) I found form and areolation of the leaves to be variable (fig. 1).

Fig. 1. *Papillaria nigrescens* (Hedw.) Jaeg. Branchleaves of one plant of the type material.

This type material, moreover, proved to be provided with some filiform microphyllous branchlets, which is all the more noteworthy as the presence of microphyllous branchlets has sometimes been regarded as a diagnostic character of *P. appressa*. I examined a specimen sent to me on loan by the Munich Herbarium; it has been labelled in an old handwriting: "Hypn. appressum Hrsch. Minas Geraes" and in pencil was added: "Neckera appressa CM v. Muell. II, 136"; some distance apart from this was written: "An C. Müller". I suppose that this must be the type material, or at least part of it, as this species was as far as I know only once again, and at a much later date, collected in Minas Geraes (Wainio, BROTHERUS, 1891). I choose this specimen as lectotype. The material consists of a few stems with some branches and a very small number of microphyllous branchlets. In the original description by HORNSCHUCH (1840) nothing is said on these branchlets. MUELLER (1851) does not mention their presence either. He states that *P.* (*Neckera*) *appressa* is very similar to *P. nigrescens* but that the stems are thicker and of a different colour and that the leaves are wider. In a note he writes: "Sterilis nota quidem, sed a N. nigrescente foliis certe distans". He saw only the type specimen of MARTIUS from Minas Geraes, probably the material cited above. MITTEN (1869) is the first who mentions: "apicibus ramusculis filiferis". He cites several specimens, including that of MARTIUS and states at the end of the description: "A M(eteorium) nigrescente foliis latioribus acumine breviorem dorso minus distincte plicatis recedit". There is indeed a rather striking difference in shape between typical leaves of the two type specimens (fig. 2 and 3).

The leaf cells of *P. appressa* are said to be shorter than those of *P. nigrescens*. HORNSCHUCH describes the cells as: parallelogrammis versus margines folii oblongis minimis". I fail to see any difference between the cells of *P. nigrescens* and *P. appressa* (Fig. 2).

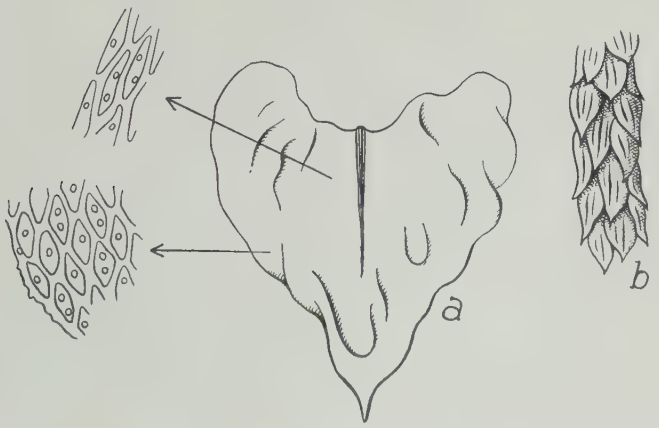

Fig. 2. *Papillaria appressa* (Hornsch.) Jaeg. (type material). *a.* Branch leaf with cells of different parts enlarged. *b.* part of dry branch.

The shape of the cells is very variable. In many Suriname specimens of *P. nigrescens* examined by me, the variation in leaf outline, in cell shape and in the number of microphyllous branchlets is nearly un-

Fig. 3. *Papillaria nigrescens* (Hedw.) Jaeg. (type material). *a.* Typical branchleaf with cells of different parts enlarged. *b.* part of dry branch.

limited. I have little doubt that critical examination of a larger number of specimens collected in different countries will show the presence of transitional forms between the two species. However, it seems premature to reduce *P. appressa* to synonymy; the decision is better left to a monographer. Nevertheless, if one wants to retain the name *P. appressa*, it would be wise to disregard characters like the presence of microphyllous branchlets and the shorter leaf cells and to restrict the name to specimens with the typical leaf form.

It should be noted here that the type material of P. nigrescens is a mixture. As is shown in fig. 4 (a photograph taken from the type specimen) the sterile plant at the right is separated by a pencil line from the left ones, which are fruiting. The plant at the right belongs to another species which is now usually called *P. imponderosa* (Tayl.) Broth. It is not known who drew the pencil line, and I could not find anything on this topic in the literature. The description of Hedwig (1801) is very short, and he does not mention the capsules. He cites the short description by Swartz (1788), who afterwards (1806) gave a long description including the capsule. Although one could defend the opinion that the figure and description given by Hedwig fit *P. imponderosa* better than *P. nigrescens*, I rejected the specimen of *P.*

imponderosa and choose the plants on the left as lectotype of *P. nigrescens*. In this way continuity in the use of the name is safeguarded and confusion avoided.

Fig. 4. *Papillaria nigrescens* (Hedw.) Jaeg. Photograph of the type specimen (S-PA).

Leucodontopsis floridana (Aust.) E. G. Britt.

A paper by THÉRIOT (1925) on this species, in which he gives a description and a figure of a capsule with peristome, has apparently been overlooked for "sporophyte unknown" has remained part of all descriptions published afterwards. THÉRIOT also describes two varieties, one of which replaces *L. horeana* R. et C. These varieties and the capsule, which are preserved in Paris (PC), interested me very much. However the capsule was a great disappointment for a re-examination showed that the branch with the capsule does not belong to *Leucodontopsis floridana* but to *Sematophyllum caespitosum*!

Among several Suriname collections of *Leucodontopsis floridana* I found an old capsule without peristome. I shall give a description here, although nothing can be said with regard to the peristome, operculum and calyptra.

Perichaetial leaves lanceolate, plane; inner ones filiform. Seta 4 mm long; theca ovoid cylindric, 1,7 mm long. (fig. 5).

Here, as in many other species with vegetative propagation, the development of the gemmae seems to suppress sexual reproduction. The same holds true for *Papillaria nigrescens*, which is seldom found with capsules but very often with microphyllous branchlets (compare European mosses like Isopterygium elegans, Aulacomnium androgynum, etc.).

Fig. 5. *Leucodontopsis floridana* (Aust.) E. G. Britt. Perichaetium and old, deoperculate capsule.

I cannot agree with Thériot on the status of his varieties. He himself already states that the variations on which they are based may occur on the same plant. And indeed, several of the leaves found on a branch of the type of his *var. latifolia* prove to be of normal shape. I did not see the type specimen of *L. floridana*, but I examined several specimens collected and identified by Miss Britton, who discovered the identity of *L. plicata* R. et C. and *Neckera* (*Pilotrichum*) *floridana* Aust.

When we measure leaves of the normal form of *L. floridana* the ratio between length and width proves to vary from 5 : 2 to 5 : 1. In typical *latifolia* leaves this ratio is 2 : 1, but a ratio of 5 : 2 and intermediate ones are also met with. I found some *latifolia* plants among a larger number of Suriname specimens, but on these plants normal leaves too occur. It seems better to withdraw this variety as well as the *var. gracilis* Thér. (syn. *L. horeana*). *L horeana* indeed is identical with *L. floridana;* the description by Renauld and Cardot (1895) already suggests this reduction. The type material (PC) shows leaves with very narrowly recurved margins. In my opinion this will be due to ecological circumstances, especially a greater humidity. The normal plants of *L. floridana* occur in relatively dry habitats (savanna bush, plantations, etc.).

I wish to thank Dr O. H. Selling and Dr H. Persson (Riksmuseet, Stockholm), Dr K. Süssenguth (Munich), Dr R. Heim and Mme Dr S. Jovet-Ast (Paris) for sending me on loan the specimens cited above, and to Dr E. B. Bartram for his critical remarks. Thanks are also due to the Netherlands Organisation for Pure Research (Z.W.O.) for making it possible to me to stay for six months in Suriname in order to study the mosses on the spot.

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PECTASE IN DOYENNÉ BOUSSOCH PEARS AND CHANGES IN THE QUANTITY OF THE ENZYME DURING DEVELOPMENT

BY

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Introduction

The evidence for the occurrence of pectase in apples and pears is somewhat confusing. According to Smock and Neuberg (1950) the presence of the enzyme in apples has never been proved satisfactorily. Kertesz (1951) states that he and his collaborators could not prove its occurrence in the same fruit. Neither apples nor pears are mentioned by Matus (1948) in a detailed survey of the literature on the occurrence of pectase in plants. Eggenberger (1949), on the other hand, is of the opinion that proof of the occurrence of the enzyme in these fruits is available in the older work of Mehlitz with Scheuer (1934) and Maass (1935). In the experiments of these investigators strong gels were formed when sap from cherries and some berry-fruits was added to 2 % apple-pectin solutions. For apples and pears, however, only small gel fragments and precipitates were found in prolonged experiments where other factors could have influenced the results. Yet in the still earlier work of Sloep (1928) and of Bertrand and Mallèvre (1894, 1895) considerable gel formation with apple extracts was obtained and later the same experimental results were published by Tserevitinov and Rozanova (1934). An explanation for this diversity of opinion as to the occurrence of pectase in fruit is given elsewhere (Weurman, 1952). Recently Pollard and Kieser (1951) measured the activity of pectase in apples by determining the methanol liberated by the enzyme but apart from the unconvincing experiments of Mehlitz and co-workers mentioned above, no other information on the occurrence of pectase in pears has appeared.

In the course of experiments to investigate the changes in pectin in pears during development and ripening it became of interest to consider the possible interference of pectase with these changes.

Methods

The presence of pectase in Doyenné Boussoch pears was demonstrated by the formation of gels when extracts of the fruit were added

to pectin solutions. The enzyme could be precipitated by addition of $(NH_4)_2SO_4$ to these extracts and the activity of the dried preparation measured titrimetrically.

Changes in the quantity of the enzyme in the fruit during the development were estimated by recording the increase in the viscosity of extract-pectin mixtures. The following procedure was followed.

Samples of at least 15 pears were picked at various dates during the development. The fruit was washed, peeled, cored, grated and ground in a mortar with quartz. To 15.00 g of the pulp 52.5 ml of 0.1 mol Na_2HPO_4 was added so as to raise the pH of the mixture to 8 and thus loosen the adsorption of the enzyme on the cell structure (cf. a.o. MacDonnell Jansen and Lineweaver (1945). The suspension was left for $^1/_2$hr at 30° C, centrifuged and the supernatant filtered. 20.0 ml of the filtrate (extract 'E') was well mixed with 35.0 ml of 0.25 % B.P. pectin * solution in

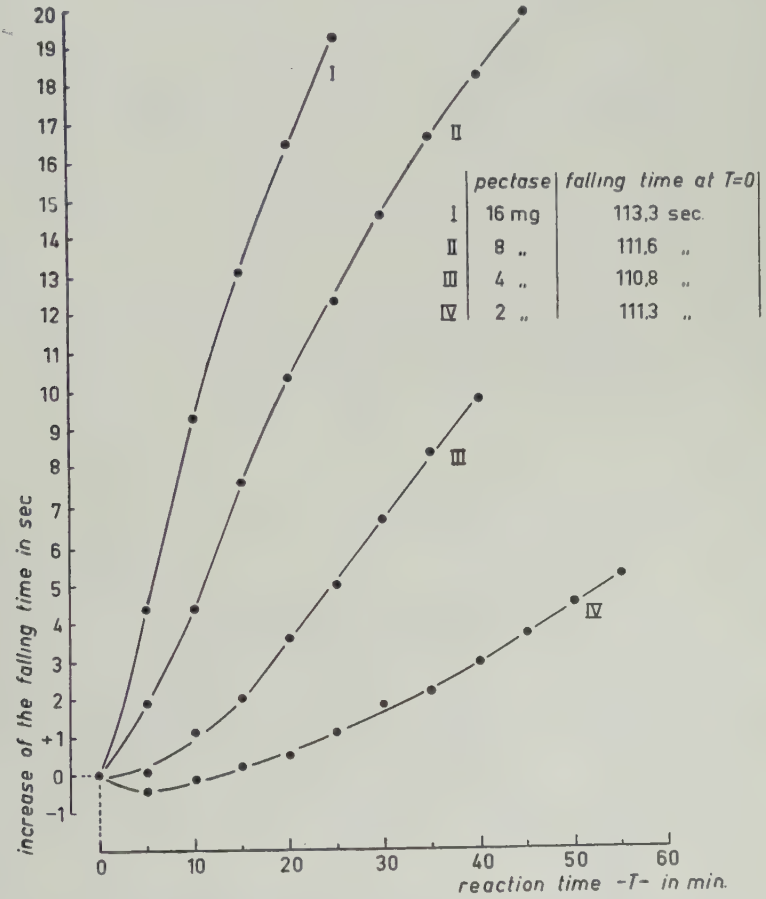

Fig. 1. The increase in viscosity of a pectin solution caused by pectase 50 ml 0,20% B.P.-pectin in 0,067 mol. phosphate buffer pH 6,0; 5 ml distilled water containing different amounts of pectase. Temp. 25° C

* Properties of the pectin, indicated by 'B.P.' are given elsewhere (Weurman, 1954).

0.067 mol phosphate buffer pH = 6.0. The pH of the reaction mixture was 6.55 ± 0.05; no change in the pH took place during the experiments. The increase in viscosity was measured using a Hoeppler falling ball viscosimeter at 25.00 ± 0.02° C. The results of the experiments are presented in the form of graphs in which the increase in the falling time of the ball indicating the increase in viscosity, is plotted against time. Control experiments were carried out with boiled extracts.

It is known that the increase in viscosity caused by the action of pectase on pectin is influenced by cations of higher valency, especially Ca- ions. The enzyme itself is activated by these ions in a complicated way (cf. a.o. LINEWEAVER and BALLOU (1945), MACDONNELL a. cow. (l.c.), PITHAWALA, SAVUR and SREENIVASAN (1948). Quite apart from their action on the enzyme, they play a role in gel formation, as was

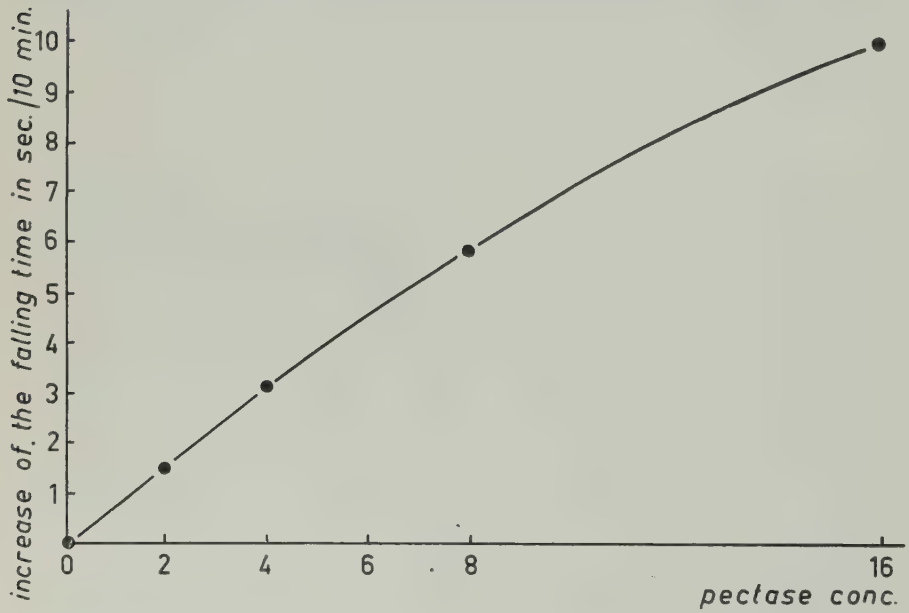

Fig. 2. The relation between the maximum increase in viscosity of a pectin solution per unit of time and the amount of pectase added to the medium. Data from graphs of fig. 1; explanation in text

pointed out clearly by KERTESZ (1951, p. 361). Because of these complications, objections can be made against the viscosity method when it is used to study the kinetics of the enzyme reaction. When used however only with the object of assessing the total activity of the pectase present in extracts, the method, besides being sensitive and consuming little time, is extremely useful as is shown in the following experiments.

Different quantities of pectase, prepared from orange flavedo according to MACDONNELL a. cow. (l.c.) are dissolved in 5 ml distilled water and added to 50 ml samples of an 0.20 % B.P.-pectin solution in 0.067 mol phosphate buffer pH 6.0. The increase in viscosity is measured following the method described above; the results are presented in Fig. 1. Some of the complications mentioned find ex-

pression in the form of the curves for which it will be diffiuclt to gvie a satisfactory kinetic explanation. When however, as is done in Fig. 2, the tangents of the straight part of the curves are plotted against the concentration of pectase in the mixture, it is evident from the resulting graph that the enzyme can be estimated quantitatively when the concentration is not too high.

Experimental results

a. The presence of pectase in pears and the isolation of the enzyme

The presence of pectase in extracts of pears picked in the early stages of development can easily be shown by the formation of gels. When 25 ml. extract "E" from pears picked in the middle of June was added to 100 ml of a 1 % B.P.-pectin solution, a strong, firm gel set after 3 hrs. With extracts of pears of later stages of development the setting of the gels was delayed while at the same time the firmness of the gels decreased. No gel formation was found any more with extracts of pears picked after the middle of August.

Although only a very weak pectase activity can be expected to exist in these fruits, it was found still possible to isolate the enzyme from pears picked in the beginning of September and kept in cold storage (2° C) for some time. Using the method of MacDonnell a. cow. (l.c.) 500 mg air-dry pectase was obtained from 400 g. tissue residue after centrifuging the pulp of these pears. The activity, measured by titration of the acid liberated from pectin by the enzyme following the method of Kertesz (1951; p. 362; 20° C i.s.o. 30° C) was found to be 12.0 P.M.U. (pectin methyl esterase units; Kertesz, 1951 p. 364 and Kertesz, 1937).

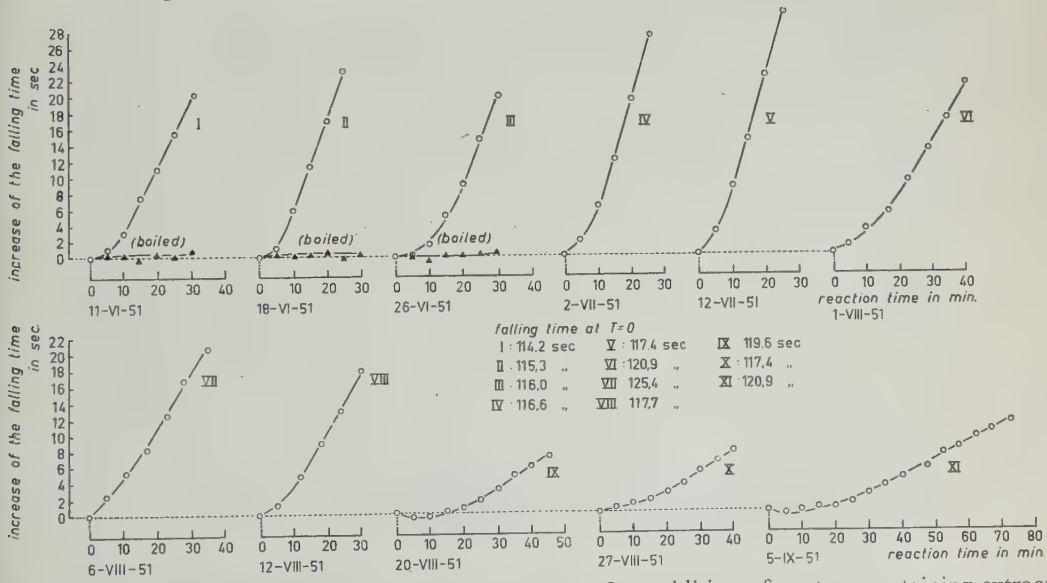

Fig. 3. The increase in viscosity of pectin solutions after addition of pectase containing extracts from pears. 35 ml 0,25% B.P.-pectin in 0,067 mol. phosphate buffer pH 6,0; 20 ml extract. Temp. 25° C

b. Changes in the activity of pectase during the development

By measuring the rate of increase in viscosity of pectin-extract "E" mixtures according to the method described above, the changes in the activity of the pectase in the fruit during its development were investigated. The results of the experiments are given in fig. 3. It is seen that the activity of the pectase in young fruits is much greater than in older ones since the curves are much steeper in the case of the former.

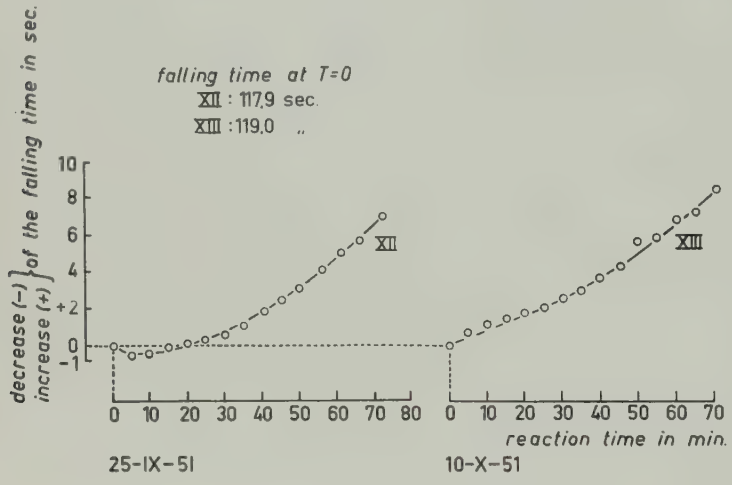

Fig. 3a. Explanation as fig. 3

In fig. 4 this is shown more clearly by plotting the gradient of the straight part of the curves against the date of picking; the activity is high in the early stages of development, starts to fall off rapidly in the second half of July and straightens out to a very low level at about two weeks before the commercial picking time (early September).

In this respect it might be of interest to mention the results of some

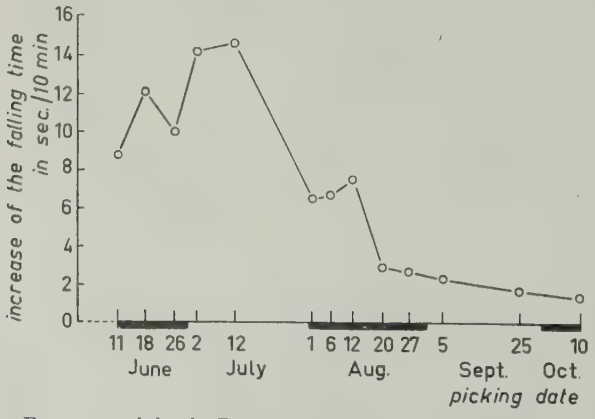

Fig. 4. Pectase activity in Doyenné Boussoch pears during development

experiments in which the behaviour of the enzyme was investigated in fruit kept in storage at low temperature (2° C) and in fruit ripened directly after picking at a date where the pectase activity was still at an intermediate level.

It was found that the activity of the enzyme did not change appreciably after picking either on ripening or storage; the activity level of the enzyme in the fruit at the moment of picking thus seems to be stabilized by the separation from the tree. In fig. 5 experimental data

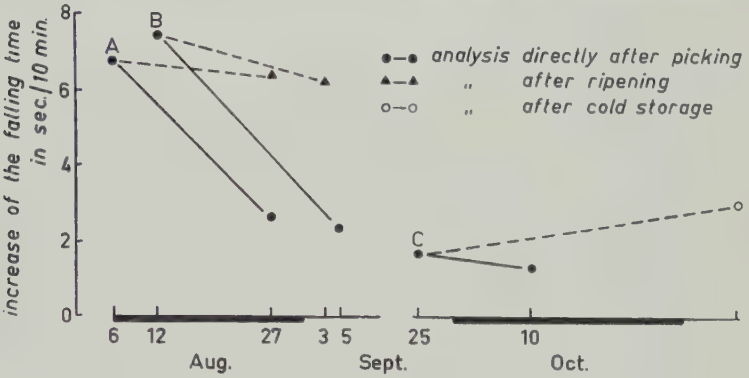

Fig. 5. Changes in the activity of pectase in pears during their development and after storage (dotted lines)

are presented. For comparison the decrease of the activity in fruits left on the tree is given in the same graph.

It is suggested that a possible explanation may be found here for the difficulties encountered with the ripening after cold storage of pears picked in too early stages of development. Confirmation of the experimental results however is considered desirable.

Discussion

In the preceding paragraphs it was shown that the rate of increase in viscosity of pectin-extract mixtures changed during the development of pears and it was concluded that the activity of the pectase present in the fruit changed accordingly. As mentioned above, the character of the reactions however on which the method of determination is based is rather complex. Therefore it should be considered whether changes in the amount of Ca in solution in the fruit might not have produced the results of our experiments.

In fig. 6 the influence of $CaCl_2$ added in different amounts to a pectin-pectase solution on the increase in viscosity is shown. The quantity of pectase used in these experiments is chosen such that the rate of increase in viscosity when no Ca is added is comparable with the rate found in fruit picked at the end of September. It is seen that the addition of Ca to the reaction mixture produces an increase in the rate. In fig. 7 this increase is plotted against the $CaCl_2$ added.

We may assume that the total amount of Ca per unit weight of fruit does not change considerably during the development. If our

results were caused by changes in the concentration of the Ca-ions in the extracts a low amount of soluble Ca should be present in the fruit in which a low "pectase activity" was found and vice versa.

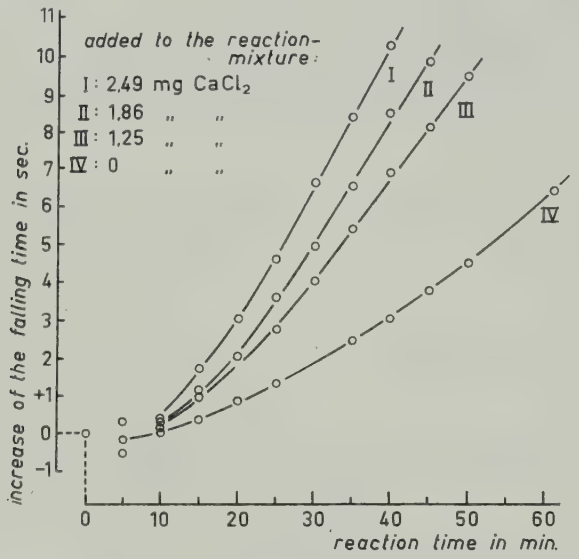

Fig. 6. The effect of Ca on the increase in viscosity of a pectin solution caused by pectase
50 ml 0,20% B.P.-pectin in 0,067 mol. phosphate buffer pH 6,0; 5 ml distilled water containing 3 mg pectase; 5 ml $CaCl_2$ solution containing different amounts of the salt. Temp. 25° C

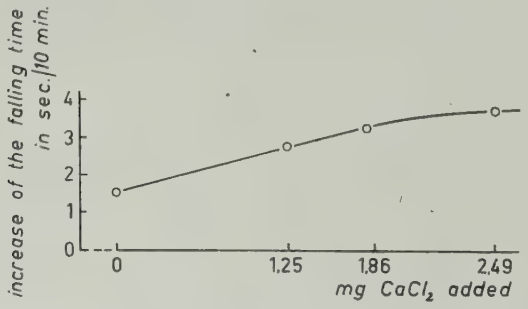

Fig. 7. The relation between the maximum increase in viscosity of a pectin solution per unit of time caused by pectase and the amount of Ca added to the medium. Data from graphs of fig. 6

In pears picked at 5/IX and kept in cold storage for about four months (presumably showing a low pectase activity) soluble, insoluble and total Ca was determined as oxalate in the ash by titration with $KMnO_4$. In the extract "E" from 100 g pulp 4.4 mg Ca was found (soluble Ca); in the residue 2.6 mg (insoluble Ca) and in 100 g pulp 7.2 mg (total Ca). Thus, even in old fruit with a low pectase activity most of the Ca is present in solution.

In the experiments to determine the activity of the pectase during the development the amount of extract "E" taken was derived from approximately 5 g of pulp and therefore contained only 1/20 of the amounts of Ca found above. From these considerations and the results presented in fig. 7 and fig. 4 it is clear that the changes in the rate of increase in viscosity during the development cannot be attributed to changes in the Ca-content of the extracts used in the experiments.

Summary

The presence of pectase in Doyenné Boussoch pears was demonstrated by gel tests and the activity of the isolated enzyme was estimated titrimetrically.

By measuring the rate of increase in viscosity of mixtures of pectin solutions and pear extracts, changes in the pectase content of the fruit during the development were investigated.

The activity of the enzyme was found to be high in the young fruit, then to start falling off rapidly at about the middle of July and to straighten out to a low level some two weeks before the commercial picking time.

Possible changes in the Ca content of the extracts used in the experiments were shown not to be responsible for the results obtained.

The fact that the pectase content of the fruit does not decrease after picking as it does when left on the tree suggests an explanation for some of the difficulties encountered with the ripening of pears after cold storage.

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PECTINASE IN PEARS

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Introduction

During the ripening of many fruits the amount of insoluble pectin in the tissue decreases while the quantity of the soluble form increases. In the last stages of the ripening process the soluble pectin itself is broken down to galacturonic acid.

However, with the exception of the tomato (McCOLLOCH and KERTESZ, 1948; idem 1949) hardly any experimental evidence has been brought forward to explain the nature of these transformations. JOSLYN, MIST and LAMBERT (1952) presented some evidence for the occurrence of pectinase (P.G.) in benzoated apple juice stored at 0° C for one month. JOSLYN and SEDLEY (1940) found a marked decrease in the estimated amount of pectin present in the pulp of apples and citrus fruit when comparing the amount directly after preparation and after leaving the pulp for definite periods of time. They attributed this decrease to pectinase, present in the pulp. Objections were made against the method used since micro-organisms (KERTESZ, 1951, p. 342) or, possibly, pectàse (McCOLLOCH and KERTESZ, 1949) could have produced the same experimental results. In fact, since many attempts to prove the presence of pectinase in apples by reductometric methods have been unsuccessful and it was found that pectin can be broken down *in vitro* to its basic units by ascorbic acid with or without the addition of peroxides to the reaction mixture, KERTESZ (1943) tentatively proposed a non-enzymic mechanism to explain these transformations. Elsewhere WEURMAN (1953) has shown the presence of a thermolabile inhibitor for pectinase in extracts from pears. The inhibitor could be isolated and some of its properties were studied. It is considered that any pectinase, if present in the fruit, would be indetectable in the presence of the inhibitor. At the same time it was found that at low pH values pectinase was more strongly adsorbed on solid cell material than the inhibitor (unpublished data).

It was decided to use this difference to try and prepare extracts and pulp residues in which the ratio of enzyme and inhibitor was shifted in favour of the former and thus to prove the presence of pectinase in the fruit.

The presence of pectinase demonstrated viscosimetrically

To prove the presence of pectinase in pears the property of the enzyme to lower the viscosity of pectic acid solutions was used (Rahman and Joslyn, 1953; Mottern and Hills, 1946; Deuel and Weber, 1945; Weitnauer, 1946; Jermyn and Tomkins, 1950).

The pulp of Doyenné Boussoch pears of different stages of ripeness, prepared by grinding the grated tissue down in a mortar with quartz, was brought to pH 7.5 by the addition of 0.5 N NaOH. The adsorption of the enzyme on cell walls is thus weakened. The pulp was filtered through silk cloth and the filtrate left overnight after the addition of toluol (Hills, Ogg and Speiser, 1945). It is essential to leave the crude filtrate for some time. The pectase present in the filtrate (Weurman, 1954) was found to lower the pH by almost two units, usually causing gel formation and thus indicating that a marked demethoxylation of the pectin had taken place. If the methoxyl content of the pectin is not reduced to as small a value as possible the pectase would interfere in the following experiments by raising the viscosity of the mixture and might thus mask the decrease in viscosity caused by any pectinase present. The filtrate was again adjusted to pH 7.5 and was filtered through paper under weak suction. Samples of the filtrate were then added to pectic acid solutions pH 4.0 and the changes in viscosity of the reaction mixture taking place during the next 30 min at 25.00 ± 0.02° C were recorded with the use of a Hoeppler viscosimeter.

In a number of experiments a marked decrease in viscosity was found indicating that pectinase was present in the filtrate. A representative example of such an experiment is given in Fig. 1. The change in the viscosity is expressed as the change in the falling time of the ball

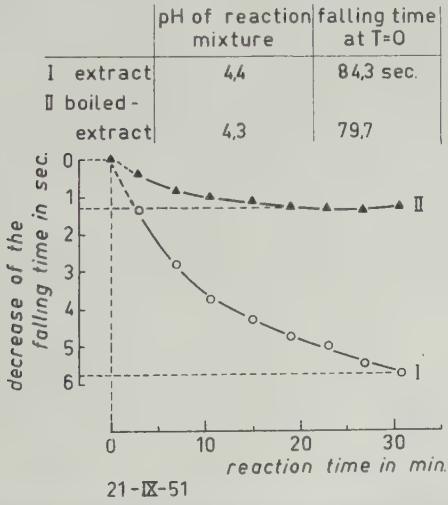

Fig. 1. The effect of an extract from pears on the viscosity of a pectic acid solution. 30 ml 2% M.P.Z.-pectic acid solution in water, pH 4.0; 20 ml pear extract; temp. 25° C.

in the viscosimeter tube and is plotted against the reaction time. The decrease still found with the boiled extract can be attributed to salt effects.

In other experiments with fruit of a different degree of ripeness no decrease in the viscosity of the reaction mixture took place (Fig. 2) and it was considered of interest to investigate the relation between the occurrence of a measurable pectinase activity and the degree of ripeness of the fruit.

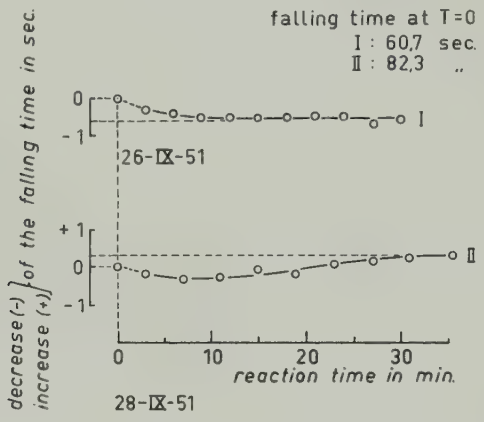

Fig. 2. The effect of an extract from pears on the viscosity of a pectic acid solution. Experimental conditions as Fig. 1.

Extracts were prepared from fruit of which the degree of ripeness was assessed accurately in advance and experiments were carried out in the way described above. The results are presented in Table I. It is seen that the presence of pectinase in the fruit could only be demon-

TABLE I

Relation between the occurrence of detectable pectinase activity and the degree of ripeness in pears

Decrease in viscosity (+) no decrease (—)	Degree of ripeness		Colour of the fruit
—	1	unripe	green
—	2	„	light-green
—	3	almost ripe	greenish light-yellow
—	3	„ „	„ „ „
+	4	ideal ripe	light-yellow
+	4	„ „	„ „
+	4	„ „	„ „
+	4	„ „	„ „
+	4	„ „	„ „
+	4	„ „	„ „
+	4-5	„ „	light-yellow:ellow
—	5	just over ripe	yellow
—	6	over ripe	orange-yellow

strated in a very short period of the ripening process when the fruit was considered "ideal ripe." This suggests a connection between the pectinase activity and the well known fact of the very short period of optimum consumption quality in pears.

We are of the opinion that a greater amount of pectinase is present in the extracts than is suggested by the rate of the decrease in viscosity found in the experiments, for it should be remembered that in these extracts pectinase inhibitor is present as well. It has been shown previously (WEURMAN, 1953) that addition of increasing quantities of the isolated inhibitor to mixtures of pectic acid and commercial pectinase caused an increasing inhibition of the enzyme up to a certain maximum. Beyond this the residual breakdown of the pectic acid was not influenced by further additions of inhibitor. It may well be that only such residual breakdown was found to take place in the experiments presented. An experiment, shown in Fig. 3 favours this

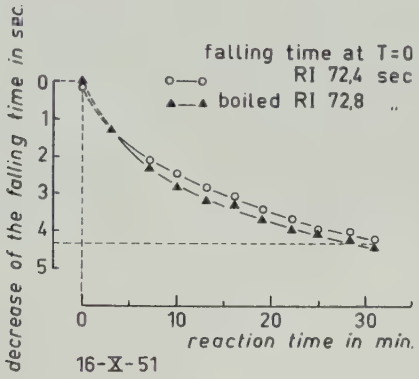

Fig. 3. The effect of an addition of pectinase inhibitor, RI, on the decrease in viscosity of a pectic acid solution caused by pear extract.
30 ml 2% M.P.Z.-pectic acid solution in water, pH 4.5; 20 ml pear extract; 5 ml solution containing 50 mg RI; temp. 25° C.

suggestion. Active and heat-deactivated pectinase inhibitor was added to a mixture of pectic acid and pear extract. The extract was found to contain pectinase as shown by the decrease in viscosity, but no difference in the rate of the decrease was found whether active or deactivated inhibitor was added indicating that a maximum inhibition had already taken place in the mixture. However, another explanation is possible for the fact that the pectinase activity in the extracts is not affected by the addition of inhibitor. As was shown previously (WEURMAN, 1953) the activity of the "depolymerase" prepared from tomatoes according to MCCOLLOCH and KERTESZ (loc. cit.) was not influenced by the pectinase inhibitor isolated from pears and it thus might well be that the pectinase from pears is identical with the tomato-depolymerase.

The presence of pectinase demonstrated reductometrically

The presence of pectinase in pears was also demonstrated by measuring the increase in reducing power of pectin solutions left in contact with the pulp. It was considered advisable in these experiments to first remove as much of the enzyme inhibitor as possible.

Finely minced pulp of healthy, over-ripe Doyenné Boussoch pears was centrifuged, the residue stirred in 5 times its volume of distilled water and the pH brought to 3.8 by adding small amounts of 0.5 NHCl. The suspension was again centrifuged and two samples (P and W) of the residue, weighing 80.0 g were taken. Sample P was mixed with 130 ml of a solution of purified pectin (2,25 g Z.M.P.-pectin in 250 ml distilled water; for properties of the pectin see Weurman, 1953), 1.00 g sodium benzoate, 2.60 ml 0.5 NHCl to bring the pH to 4.0 and 3.20 ml distilled water (final volume 80.0 + 135.80 ml). Sample W, the blank of the experiment, was mixed with 130 ml distilled water, 1.00 g sodium benzoate and 5.80 ml 0.5 NHCl, by which again the pH was brought to 4.0 (final volume 80.0 + 135.80 ml).

Both samples were divided in two equal parts (a and b). P.a. and W.a. were centrifuged directly after preparation and the reducing power of 5 ml samples of the filtered supernatant was measured according to Willstätter and Schudel (1918). P.b. and W.b. were left at 30° C for 3 days; the pH was kept constant by the addition of 0.1 N NaOH while distilled water was added to keep the volumes equal. After 3 days the reducing power was measured as in the other samples. The results of the determinations (average of 4 closely agreeing determinations) are given in Table II.

TABLE II
Explanation in text

Sample	Time (days)	Substrate	0.1 N $Na_2S_2O_3$/5ml filtrate
P.a.	0	pectin	3.73 ml
P.b.	3	„	3.35 „
W.a.	0	water	3.77 „
W.b.	3	„	3.76 „

It is seen that an increase in reducing power was only found when purified pectin was added to the reaction mixture (P.a., P.b.). Since 1 mg galacturonic acid is equal to 0.097 ml 0.1 N thiosulphate in reducing power, 3.8 mg galacturonic acid was formed in 5 ml filtrate of the reaction mixture originating from approximately 35 mg pectin (water content of the original centrifuged residue taken into account). So 10 % of the pectin added to the tissue residue was broken down to galacturonic acid by the pectinase present in the fruit tissue.

It should be mentioned that the precipitates formed on addition of acetone to the filtrates of P.a. and P.b. were repeatedly found to differ markedly from each other. The breakdown of pectin was thus easily perceptible in P.b. These observations are very convincing for

the experimentor working in this field as an indication for the presence of an active pectinase in the medium (KERTESZ, 1951, p. 343).

SUMMARY

Finely minced tissue of Doyenné Boussoch pears was either extracted with dilute acid or treated with alkali prior to extraction to obtain pulp residues or extracts in which the ratio of pectinase inhibitor to pectinase has been changed in favour of the enzyme. The presence of pectinase in either the pulp residue or the extract was then demonstrated by reductometric and viscosimetric methods.

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GAS-EXCHANGE IN THE VESICLES (AIRBLADDERS) OF ASCOPHYLLUM NODOSUM

BY

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CONTENTS

Introduction

A study was made of the exchange of gases through the vesicle-walls of the brown alga *Ascophyllum nodosum* under laboratory conditions.

The gas filled air-bladders or vesicles of *Fucaceae* and *Laminariaceae* have been studied by a number of botanists. The development of the vesicles was described by Reinke (1876), who observed that in *Fucaceae* a vesicle originates in a predisposed place on the thallus, e.g. in the form of a flat disc. The anatomy of the young vesicle is the same as in the normal thallus. On the outside a number of layers of closely packed assimilating parenchyma cells (cortex) are observed. On the inside a pith of hyphae is found, to which a re-enforcing or translocating function is ascribed. Between the hyphae, intercellular cavities are found, filled with mucus. The cavities are particularly spacious in the places where the vesicles will be formed and gasbubbles are secreted in these cavities. The bubbles enlarge and unite and thus form the vesicles. The vesicle, then, has the same outer layers of closely packed parenchyma cells, while on the inside there are the living remains of the hyphae, which are fibrous and loose. The vesicle should thus be considered as a closed intercellular space.

A question arises as to why the gas is secreted in the intercellular cavities and not in the interior of the cells. According to Henry's law, the solubility of gases in fluids is proportional to the pressure. In the

interior of a cell under osmotic turgor much more gas can be kept in solution before saturation takes place and a bubble will be formed. On the outside of the cells, under normal pressure, saturation will take place at lower concentrations. When gases can diffuse through the walls of the cells, bubbles will form in intercellular cavities or on the surface of the plants and not in the interior of the cells. This fully agrees with observations made on submersed plants forming oxygen at photosynthesis.

The composition of the gas in the vesicles was studied by Wille (1889), Zeller and Neikirk (1915), Langdon (1917), Langdon and Gailey (1920), Colla (1930–1935), Damant (1936), Valence and Coult (1951). Oxygen and nitrogen, though found in varying concentrations, approach the composition of normal air. Carbon dioxide is present only in very small amount, the concentration being somewhat higher during the night. An interesting observation was made by Langdon (1917) and by Langdon and Gailey (1920), who found the occurrence of carbon-monoxide in the large vesicles of *Nereocystis*. Though there are indications that carbon-monoxide may also occur in other species, its presence has not been adequately proved. According to Langdon and Gailey (1920) the carbon-monoxide in *Nereocystis* is also formed during darkness and is thus a product of metabolism. The inside of the vesicles is sterile, as was found by Rigg and Henry (1935), and the carbon-monoxide cannot, therefore, be formed by bacterial action. In addition to these gases there is a small amount of water vapour.

It is commonly held that oxygen is formed in the vesicles by photosynthesis and that an interchange of this gas and the gases of the surrounding medium takes place. The normal composition of the air is thus approached. Valence and Coult (1951) observed that the oxygen content rarely decreases much below 20 %. Carbon dioxide from which the oxygen is formed is present in the seawater or is formed by respiration. The solubility of carbon dioxide is, however, so much higher than that of oxygen and nitrogen that it is only present in very small amounts in the gas of the vesicles. The carbon dioxide that may be formed during the night will either quickly dissolve in the water of the tissues and diffuse to the outside or it will disappear at photosynthesis.

Moravek (1929) applied various dilutions of seawater to both sides of the vesicle wall of *Nereocystis* and observed that chlorides were translocated in either direction along the concentration gradient.

In the present investigation vesicles of *Ascophyllum nodosum* were placed under different conditions of light and darkness and in gas-free or aerated seawater. The change in volume and composition was then observed and an explanation was given of the phenomena that occur under natural conditions. Further the permeability of the wall for gases, water and other substances was studied.

The experiments were carried out at the Laboratory of the Zoological Station at Den Helder. The author is indebted to Prof. Dr W. H. Arisz for his criticism and to Mr H. Postma for his help with the analysis.

Material and Methods.

Ascophyllum nodosum was collected at low tide on the stony slopes of Nieuwediep harbour near Den Helder. The plants keep fresh for a few days in a moist and cool place in a basket. Storage for longer periods was possible in an aquarium building with a glass roof.

The experiments were carried out at room temperature or in a refrigerator. A suitable gasfree medium was prepared by bottling seawater when still boiling.

The approximate decrease of the gas in the vesicle can be judged from the occurrence of dimples or deflation. Old vesicles, however, have thick walls that are not flexible and often may not display any dimples or deflation when gas is lost. Therefore young vesicles were used in the experiments.

The concentration of oxygen was determined with the so called Jordan gas pipette (1927). The following solution was used as an oxygen adsorbing agent.

NaOH or KOH 4 molar	11,2 cc
Distilled water	40 cc

To this is added 10 gr $Na_2S_2O_4$ and 1 gr of Natrium-antrachinon-betasulfonate. The solution was kept under paraffin-oil. For the adsorption of carbon dioxide a one molar solution of NaOH was used.

The permeability of the vesicle walls for other substances as gases was studied by filling the vesicles with solutions. This was done by means of a calibrated syringe. The hollow needle was introduced through the flat part of the thallus (Fig. 1) and afterwards the hole was closed with a wooden clamp (Fig. 2) The other end was

Fig. 1

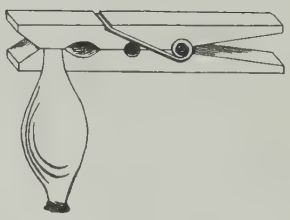

Fig. 2

dipped in paraffin and the end with the clamp was in the experiments always kept above the level of the fluid. Changes in the volume of the solution were measured with the syringe, with a correction for water adhering to the inner wall of the vesicle. In experiments where a

gain of volume is expected, the vesicles were only filled for a small part.

In control experiments dead vesicles were used. These were killed by immersion in seawater of about 80° C.

Experiments

A. Permeability of the walls

The permeability of the walls for gases can be demonstrated by keeping the vesicles in gasfree seawater. The vesicles then form dimples and become deflated after some days in darkness and subsequently seawater will enter by suction. This process occurs at about the same rate at low temperature, so it is for the greater part not due to respiration of the oxygen; it also occurs in dead vesicles. The results of the experiments are given in the following table:

Vesicles in gasfree seawater. Each batch 5 vesicles.

Condition	Temperature ° C	Treatment	Dimples after:	Deflation after:	Remarks
alive	16	darkness	1 day	2 days	some water inside
alive	3	,,	1 ,,	2 ,,	
dead	16	,,	1 ,,	2 ,,	much water inside
dead	3	,,	1 ,,	2 ,,	
Controls in aerated seawater. Each batch 5 vesicles.					
alive	16	darkness	no dimples or deflation after 2 days.		no water inside
alive	3	,,			
dead	16	,,			
dead	3	,,			

The loss of gas and the influx of water in the first series of these experiments in gasfree seawater may go so far that after 4–5 days some of the vesicles no longer float but sink to the bottom. It also follows from these experiments that water may enter in the vesicles, after a loss of gas. The walls of the vesicles must therefore be permeable for water.

This permeability for water was also demonstrated by filling the vesicles with a solution that differs from the outside solution. Water is then translocated through the walls and can be estimated by measuring the change in volume. In using tapwater against seawater the following results were obtained.

Vesicles with different concentrations at both sides.
Each batch of 3 large vesicles. Temperature 16° C.

Inside	Outside	cc used	cc after 24 hrs	Remarks
tapwater	seawater	1,0	0,6-0,7-0,8	
tapwater	tapwater	1,0	1,1-1,1-1,0	control
seawater	tapwater	0,5	0,8-0,9-1,0	
seawater	seawater	0,5	0,5-0,5-0,5	control

Various substances can diffuse through the walls. A number of vesicles was filled with a solution of 1 gram of ammoniumphosphate

per Liter of seawater. The ammonia was then ascertained in the surrounding solution after 2 days at 3° C. Moravek (1929) observed for *Nereocystis* that chlorides were translocated through the walls of the vesicles along a concentration gradient. He used diluted seawater in different concentrations.

When applying a solution of 1 gram of sodium nitrate per liter of seawater inside the vesicles, nearly all nitrate was found in the surrounding solution after 2 days The determinations were made by a method worked out by Foyn (1951) for seawater.

Eosine, which is known not to penetrate well into protoplasm, was found to diffuse from the inside of the vesicles into the surrounding medium and after 2 days a red colour was observed. The vesicles were filled with a 0,2 % solution of eosine in seawater.

The pathway of the diffusion could be followed by using potassium-ferrocyanate. A 1 % solution of this salt was used inside the vesicles in a mixture of equal parts of tapwater and seawater. The vesicles were then left for 2 days in seawater and then sections were made from the wall. These sections were immediately fixed with alcohol, to which a few drops of ferrichloride had been added. A precipitate of prussian blue was then formed in those places of the sections where the cyanide was present. The sections were then cleared with chloralhydrate in order to give more prominence to the stained parts. The internal layers displayed a dark blue colour, but more on the outside a light shade of blue could only be observed in the walls of the cells and not in their interior. So it is obvious that the diffusion went through the connecting walls and not through the protoplasm.

Sugars also diffuse through the vesicle walls. This can be demonstrated by applying equimolar solutions of sucrose and glucose on both sides. Concentrations were used which had about the same osmotic value as seawater. That is 20 % sucrose or 10,5 % glucose. After the experiment dry weight and volume were determined. All experiments were performed at 3° C in order to prevent the loss of sugar by respiration or bacterial decomposition.

Glucose molecules pass about 3 times as fast through the walls as sucrose molecules. This causes a difference in osmotic concentration between both sides. This difference will be leveled by translocation of water.

When glucose is put inside the vesicles and sucrose on the outside, up to two thirds of the volume of the solution in the vesicles disappears. In the end the solution inside the vesicles has the same percentage of dry weight as the sucrose solution on the outside. When sucrose is put inside, and the vesicles are only partly filled, the amount increases up to 2,5 times the original volume and the percentage of the dry weight in the end is about the same as in the surrounding glucose solution. Controls with the same solution on both sides did not show large changes in volume or dry weight percentage.

B. Exchange of gases

It was shown before that in the dark, vesicles in gasfree seawater give off part of their gas, so that they become dimpled or deflated. This phenomenon also occurs at low temperature or in dead vesicles and the loss of gas must therefore, in the first place, be due to a removal of the gas as a result of the low pressure of gas in the outside solution.

If, under conditions of darkness, the vesicles are brought into aerated instead of gasfree seawater, the results are different. At room temperature a slow decrease is observed, this decrease is very slow at low temperature and it is not observed in dead vesicles. The decrease in volume is connected with the use of oxygen by respiration, as can be seen from the following tables.

Loss of volume in aerated seawater in darkness
Each batch five vesicles

Condition	Temperature ° C	Vesicles with dimples after days 1 2 3 4 5 6 7	Remarks
alive	16	– – 1 2 3 3 5	after 7 days water inside
dead	16	– – – – – – –	after 7 days no water inside
alive	3	– – – – – – –	
dead	3	– – – – – – –	

Loss of oxygen in aerated seawater. Each batch 3 vesicles.
Trace of CO_2 present. All vesicles in darkness and alive.

Time	Temperature ° C	Oxygen %	Temperature ° C	Oxygen %
at start	—	22-22-23		22-22-23
after 2 days . . .	20	15-10- 8	5	15-16-16
after 4 days . . .	20	4- 4- 7	5	16-13-15

If vesicles are kept in gasfree seawater in sunlight, their gas diffuses out as a result of the low pressure outside, but new oxygen is produced by photosynthesis and the percentage of oxygen increases considerably. The results are given in the following table. A trace of sodium bicarbonate was added together with the vesicles to procure enough carbondioxide for photosynthesis.

Vesicles in sunlight and in gasfree seawater.
Each batch 3 determinations. Temp. ± 16° C. No carbon dioxide found.

Condition	Days	Oxygen %	Average oxygen %	Remarks
alive	0	21 22 21	21	full
alive	2	51 52 49	51	full
alive	3	60 68 71	69	full
dead (control)	3	— — —	—	all with dimples or deflated

The table shows that at ± 16° C the oxygen percentage in the living vesicles in sunlight may be about 70 % oxygen after 3 days in gasfree seawater. The dead control vesicles lose their gas, which is not replaced by new oxygen. As a result they will become dimpled or deflated and seawater will enter.

C. Entry of seawater

In full vesicles with a positive pressure, seawater is never found inside. This occurs only when, due to some reason or other, the inside pressure becomes negative. When much gas is lost this is due to the resilience of the elastic walls. This condition of negative pressure can be obtained under laboratory conditions, by keeping the vesicles in the dark in gasfree seawater, or even when exposed to aerated seawater in the dark for several days and at room temperature. All dimpled or deflated vesicles will then get some water inside in the end. In the beginning the water is barely perceptible, but after some days under these conditions, more and more water enters and in the end the vesicles are for the larger part filled with seawater. Then gradually the dimples will disappear and the vesicles look apparently normal, but the inside is filled with water and hardly any gas is found.

In the month of December it was observed that on newly gathered algae some of the vesicles contained considerable amounts of water. This seems to agree with our general view on this subject.

Discussion

Valence and Coult (1951), who analysed the changes in the composition of gas in the vesicles of *Fucus vesiculosis*, found the oxygen percentage may be markedly enhanced by photosynthesis. Damant (1936) observed a correlation between the pressure in the vesicles of *Ascophyllum* and the oxygen percentage. He gives values from no pressure at all with 20 % oxygen to 4 lbs per square inch with 35 % of oxygen in the gas of the vesicles. The pressure was highest in April and lowest in November. He also observed that the oxygen percentage was highest in the upper vesicles, which got most of the sunlight. In the present investigation it was found that the oxygen content of vesicles may, in gasfree seawater, amount to 70 % after several days exposure to sunlight. From these observations it can be concluded that the gas in the vesicles is conditioned by the formation of oxygen during periods of photosynthesis.

Gases diffuse through the walls of the vesicles. This can be demonstrated by keeping vesicles for a few days in the dark in gasfree seawater. The gas disappears and the vesicles become deflated and then have the shape of the hollow bowl of a spoon. The same is observed in dead vesicles or in living vesicles at low temperature, which demonstrates that loss of gas is for the major part due to diffusion.

Diffusion of gases through the wall was also demonstrated in an experiment by Damant (1936). *Ascophyllum* strands were tied to a weight and a rope and thrown in deep water. After a few hours, when the plants were again brought to the surface, it was observed that most

of the gas had disappeared and that the vesicles were deflated. This can be explained as follows. More gas can dissolve under high pressure. In the sea, however, the saturation can only take place at the surface. When such water circulates to the depth a large saturation deficit of gases will occur and all gases present will rapidly go into solution. This is according to Henry's law that the solubility of gases is proportional to the pressure. The gas from vesicles, when brought into deep water will, therefore, rapidly diffuse and dissolve in the seawater. As a consequence of this, loss of gas from vesicles in deep water will, within a few hours, be as large as the loss of gas during days in gasfree seawater in the dark.

Gases may diffuse from both sides. The composition of the gas in the vesicles will therefore always strive towards the composition of air in equilibrium with the water around the vesicles. Differences in partial pressure will thus be leveled out.

Valence and Coult (1951) observed that, even after prolonged periods in the dark, the oxygen content rarely fell below 20 %. In the authors experience, however, a considerable loss of oxygen was found under laboratory conditions and especially at high temperatures. When oxygen is used by respiration of the tissues the volume of the vesicles decreases gradually, because nitrogen goes out due to the fact that the partial pressure of the nitrogen is too high when oxygen is used. A similar phenomenon was described by Ege (1918) on the function of airstores carried by some aquatic insects. From the air, oxygen is used by the insect and the partial pressure of this gas is lowered. Then new oxygen diffuses but at the same time nitrogen goes out. The bubble, though taking in oxygen, becomes gradually smaller untill the insect has to go to the surface to replenish his supply. Carbon dioxide that is set free immediately dissolves in the water. At low temperature the store will last much longer because less oxygen is used. This was also found in the vesicles. When kept at a low temperature in the dark in aerated seawater, the time required for dimples or deflation was much longer that at room temperature. The walls of the vesicles offer some resistance and make the processes of exchange slower than in ordinary gas bubbles. However, the principle of the exchange remains the same.

Variation exists in the thickness of the walls; the older vesicles on the lower parts of the plants have, in general, thicker walls. Diffusion through these walls is slower and it takes a longer time before such vesicles become dimpled or deflated under experimental conditions. Damant (1936) claims that this may be an adaptation to places where a large difference exists between the tides. At high water too much gas will diffuse from the vesicles and this can only be counterbalanced by thick walls or by intense photosynthesis.

The walls were not only permeable for gases but also for water, salts and sugars. Water could be forced through the walls when a difference in osmotic concentration was given between both sides. When pressure in the vesicles becomes negative seawater will be sucked in. Salts diffuse through the walls, as was also observed for chlorides by Moravek

(1939) for *Nereocystis*. With potassium ferrocyanide the pathway of the diffusion could be followed through the walls between the cells and not through the protoplasm.

The pressure and composition of the gas in the vesicles, will under natural conditions, depend on the intensity of photosynthesis. This can however be counteracted by a loss of gases. Under certain conditions the loss of gases may prevail over the photosynthesis and the pressure will then become negative and seawater will be sucked in through the walls. This was observed on some vesicles in the month of December.

Summary

1. The gas in the vesicles (airbladders) of the brown alga *Ascophyllum nodosum* is conditioned by the formation of oxygen during periods of photosynthesis.

2. The walls of the vesicles were found permeable for gases, water, salts and sugar. Seawater will, however, only enter the vesicle when the pressure becomes negative.

3. An exchange of gases takes place through the permeable walls. Differences in partial pressures of gases between both sides of the vesicle wall are leveled.

4. Under natural conditions the pressure of the gas and the oxygen content depend on the intensity of photosynthesis. This is, however, counteracted by other factors.

a. loss of gases by diffusion when the partial pressure of the oxygen in the vesicles is higher than in the surrounding water.

b. loss of gases due to a saturation deficit in deeper water, e.g. at high tides. The gases then disappear into the surrounding water. Damant (1936).

c. loss of oxygen by respiration. The nitrogen then also leaves the vesicle. The formed carbon dioxide is highly soluble and thus disappears.

When these factors prevail over photosynthesis, a negative pressure may develop and seawater will be sucked in through the walls. This was observed on some vesicles in the month of December. It can also be induced under laboratory conditions.

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ACQUISITIONS TO THE MOSS AND LIVERWORT FLORA OF THE NETHERLANDS

BY

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(Received Nov. 12th, 1953)

A growing interest in the study of the bryophytes has become evident in the last decade. This interest has been strongly stimulated by the many excursions which the "Bryologische Werkgroep" has held nearly all over the country. Moreover, the fact that bryophytes are dominant in some special habitats (tree trunks, Sphagneta) has induced quite a few biologists, interested in plantsociology, to study them intensively. This revival has led to many results in the investigation of our moss flora, particularly in the discovery of a number of new and rare species.

An earlier publication (N.K.A. 57: 281, 1950) has already given a number of these and an additional number have been briefly dealt with in the periodical "Buxbaumia".

There is another circumstance which has contributed to a better knowledge of our bryophytes. The strong growth of our population has caused a great demand for arable land. Consequently, many waste lands have been reclaimed, bogs and marshes drained, brooks canalised, etc. It therefore became imperative to preserve as many of our incomparable moors, marshes, etc. as possible. Their value as future nature reserves had first to be assessed from an accurate survey of their fauna and flora. Naturally this also included the bryophytes.

It cannot be gainsaid that a general impoverishment of our moss flora has taken place in the course of the last century. Many tracts of wild land have disappeared whilst the increasing industrialisation of the country has made large areas unsuited for the growth of mosses. Many species which were quite common a century ago are now limited to a few localities e.g. *Antitrichia curtipendula* and *Neckera pumila*, while other, rarer mosses have probably become extinct: e.g. *Splachnum ampullaceum* and *Cinclidium stygium*. It is to be feared that this impoverishment will proceed at an increasing rate in the future. Nevertheless a number of new indigenes have been discovered as well as an additional number of rare and remarkable species. This publication mentions 18 new indigenous species, of which 13 are Musci and 5 Hepaticae. In addition it mentions 5 new varieties (Musci) of which 3 are new to science, and 20 new records of rare bryophytes (14 of mosses and 6 of liverworts).

SPECIES AND VARIETIES NEW TO OUR FLORA

A. Musci

Barbula gracilis Schwaegr.

Leg. A. J. H. M. van de Ven, 1–10–1949, Achtbundersweg, V6. **23**.13, Schin-op-Geul, prov. of Limburg, in a *Mesobrometum* on cretaceous limestone (Maestrichtien).

Most of the differences between *Barbula gracilis* and *Barbula fallax*, indicated by Dixon (1924), are unreliable: both have the nerve much protruding at the back of the leaves and often quite flat on the ventral side; in neither of the species there is a difference between the leaf cells on the nerve and those on the lamina; they are very short in the upper part of the leaf, more or less elongated towards the base, both on nerve and lamina. As a rule those in *Barbula fallax* remain very short to the base, while those of *Barbula gracilis* are distinctly longer near the base. In the material from S. Limburg, however, the basal cells are short, but the same was observed by me in an exsiccate from Upsala (Sweden). On the other hand, exsiccates of *B. fallax*, collected by Brotherus, proved to have much elongated basal cells!

In my opinion the most reliable differences between *Barbula gracilis* and *Barbula fallax* are the smooth leaf cells of the former in contrast to the papillose cells of the latter, and the position of the leaves: in *Barbula gracilis* straight and closely appressed when dry, erecto-patent when moist, in *Barbula fallax* somewhat twisted when dry, squarrosely recurved when moist. Generally too, the leaves of *Barbula gracilis* have a longer and narrower acumen and a nerve which is sometimes excurrent. J. J. B.

Barbula sinuosa Braithw.

Leg. J. J. Barkman, no. 3699, 10–10–1951, N. of Deventer, M6. **65**.22, prov. of Overijsel, on the base of a pollard-willow in foreland of river IJsel, flooded in winter, rel. [1] 1397.

Although Moenkemeyer (1927) includes this species as a variety of *Barbula cylindrica* (Tayl.) Schimp., I consider it distinct enough to give it specific rank; see also Dixon (1924: 215–216). J. J. B.

Bryum capillare Hedw. var. **rosulatum** Mitt.

Leg. J. J. Barkman, no. 3685, 7–8–1951, Linde valley, Oldeberkoop, K6.**15**.22, prov. of Friesland, on stumps of *Fraxinus* in moist ash-alder-coppice, rel. 1085.

The aspect of this variety is strikingly similar to that of *Rhodobryum roseum*. The leaves are crowded in a terminal rosette, squarrosely recurved when moist, shrinking, but widely spreading and not spirally twisted when dry, very large ($3\frac{1}{2}$–$4\frac{1}{2}$ mm long), with entire, recurved margins and mostly percurrent nerve. J. J. B.

[1] In this paper "rel." stands for plantsociological "relevé" (sample plot survey).

Bryum funckii Schwaegr.

Leg. A. J. H. M. VAN DE VEN: 1–10–1949, Wahlwiller, V6.**33**.42, prov. of Limburg, initial stage of *Mesobrometum* on loose cretaceous marl (Gulpens); 30–9–1949, Schiepersberg between Bemelen and Houthem, V6.**21**.23, prov. of Limburg, *Mesobrometum* on cretaceous chalk rocks (Maestrichtien).

Both samples have about 1 cm. long, very fragile, catenulate stems. All leaves are of the same size and shape, i.e. very small (0.5 mm long and nearly as broad), of a very thin texture, concave with flat, but not recurved, entire margins without border. The leaf cells are all wide, shortly hexagonal and thinwalled. The nerve is strong, reddish at base, otherwise dark green or brownish, ending in apex or excurrent in a short apiculus.

This is a calciphilous species, known from Central-Europe, so it could be expected here.

Quite a different matter is the question whether this species — like so many other species of *Bryum* based exclusively on gametophyte characters — can be looked upon as a sound species. In my opinion it is quite possible that *Bryum funckii* merely represents a variety of either *Bryum argenteum* or *Bryum caespiticium*. J. J. B.

Calliergon megalophyllum Mikutowicz

Leg. W. MEYER, April 1948, Belversven, prov. of N. Brabant.

In the last five years *Calliergon megalophyllum*, a species mentioned by MIKUTOWICZ in his Bryotheca Baltica, no. 141 (1918), has received attention from the Scandinavian bryologists TUOMIKOSKI (1937, 1940) and JENSEN (1939).

At first sight the differences between *Calliergon megalophyllum*, *Calliergon giganteum* and *Calliergon richardsonii* seemed so slight to me, that I was dubious as to the specific rank of the first named. However, through the kind intervention of Dr PERSSON I was able to send all my Dutch *Calliergon* material to Dr TUOMIKOSKI, who named one of my specimens *Calliergon megalophyllum* and sent me fine Finnish samples for comparison. The study of his material quite convinced me of the specific rank of *Calliergon megalophyllum*.

Calliergon megalophyllum differs from *Calliergon giganteum* in the absence of the numerous lateral branches with smaller leaves so characteristic of the latter, and by the smaller auricles not reaching the nerve. The nerve itself is less pronounced than in *Calliergon giganteum*. From *Calliergon richardsonii* it may be known by the longer nerve reaching the apex, the green colour, instead of a red tendency in *Calliergon richardsonii*, and the dioicous inflorescence.

Up to now *Calliergon megalophyllum* has been reported from Norway, Sweden, Finland and Russia by TUOMIKOSKI (1940), and was supposed to be boreal-continental. This new record from Holland is far to the southwest of the known localities.

The habitat of *Calliergon megalophyllum* in the Belversven is similar to most of the Scandinavian stations. The pH of the open water ranged

from 6.6–7.0 (Van Heusden, 1949). Our *Calliergon* was found along the fen margin, 1–2 dm submerged, among *Carex inflata*, *Phragmites communis*, *Schoenoplectus lacustris*, *Glyceria spectabilis* and *Sparganium minimum* in the upper herblayer, and *Menyanthes trifoliata* and *Comarum palustre* in the lower herblayer, while *Hydrocharis* was floating.

W. M.

Dicranoweisia crispula (Hedw.) Lindb.

Leg. W. D. Margadant and N. E. Nannenga-Bremekamp, herb. N.E.N.-B. no. 1079, 15–8–1950, Hoge Veluwe, prov. of Gelderland, on concrete wall.

From a single very compact, dark greyish tuft, growing about $1\frac{1}{2}$ m up an exposed concrete wall, a part was collected. This sample proved to be different from the in the Netherlands common *Dicranoweisia cirrata* in the following points: the longer narrower leaves with plane margins and the small inflated auricles. We therefore refer this specimen to *Dicranoweisia crispula*.

Geographically the occurrence of this mountainous species here is

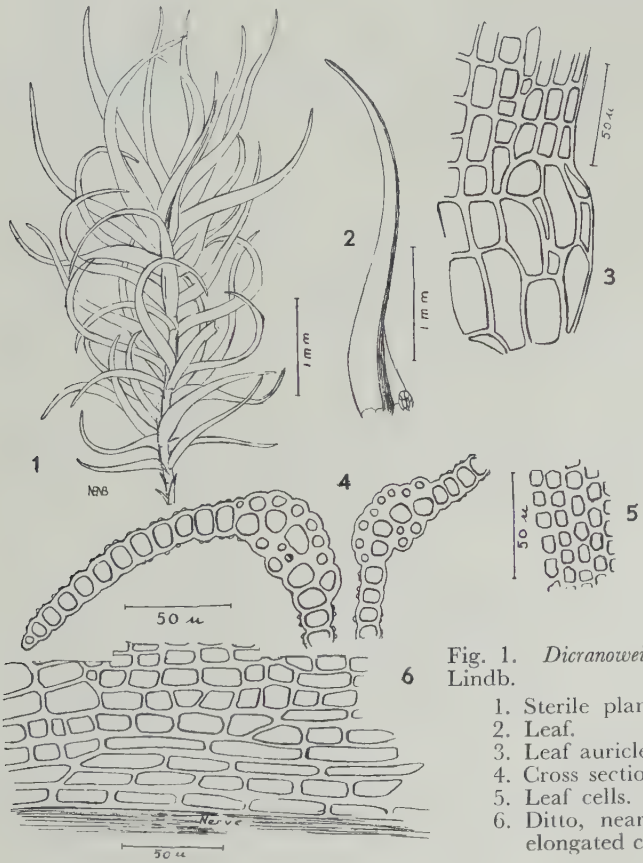

Fig. 1. *Dicranoweisia crispula* (Hedw.) Lindb.

1. Sterile plant.
2. Leaf.
3. Leaf auricle.
4. Cross sections of leaves.
5. Leaf cells.
6. Ditto, near the base, showing elongated cells next to the nerve.

remarkable; it may have been imported with the Maulbrunner sandstone, which was deposited in the vicinity of the wall, but on which our moss was not found. On the other hand it may have arrived by wind from more mountainous regions, perhaps via isolated rocks in the Northern German Plain from where there are some records too. (Fig. 1) W. D. MARG. and N. E. N.–B.

Dicranum fuscescens Turn. **var. falcifolium** Braithw.

Leg. W. MEYER, April 1950, Speulderbos, N5.**17**.24, prov. of Gelderland, on an oak tree.

This is a variable species and sometimes difficult to distinguish from *Dicranum mühlenbeckii* B. et S. The latter differs chiefly in the highly tomentose stems, the leaves more crisped when dry, strongly tubular above, and coarsely denticulate. *Dicranum fuscescens* occurs mainly in mountainous districts; in the Alps up to 2800 m altitude, on moors, rocks etc., in Scandinavia, Great-Britain, N. Germany, Belgium (practically only in the "district Ardennais") and even in Spitsbergen. The variety is much less common. W. M.

Dicranum strictum Schleich.

Leg. W. D. MARGADANT, no. 822 and N. E. NANNENGA–BREMEKAMP, no. 722, 2–4–1950, Molenbeek, Renkum, P5.**28**.23, prov. of Gelderland, in the fissures of oak bark; leg. N. E. NANNENGA-BREMEKAMP, no. 2380, 4–6–1953, Heelsum, prov. of Gelderland, on and around stumps in a beechwood.

A small *Dicranum* growing in the fissures of the bark of an old oak was collected near Renkum. There was a superficial resemblance to *Dicranella heteromalla*, but our specimens had straight leaves. Under the microscope the resemblance disappeared altogether with the presence of well developed auricles. The long, entire, brittle leaves pointed to *Dicranum strictum*. Leaf section verified this since there were no stereid cells in the nerve, as is the case in the closely allied *Dicranum viride*.

Geographically it is an interesting plant with a mainly mountainous distribution, and only sporadically encountered in low countries: there are records from the Northern German Plain, from Brittany and from England. (Fig. 2) W. D. M. and N. E. N.–B.

Grimmia apocarpa Hedw. **var. bistratosa** Barkman **nov. var.**

Leg. J. J. BARKMAN, no. 3632 (type specimen in Rijksherbarium, Leiden), 16–7–1951, Krimpen a/d Lek, P4.**41**.43, prov. of Z. Holland, on the base of a pollard-ash (South side of trunk) in marshy foreland of the river Lek, flooded twice a day by fresh water, rel. 964.

Differt laminis foliaribus bistratosis in tota parte dimidia superiore foliorum, unde per laminam unistratosam descendunt fere usque ad basim fasciae bistratosae longitudinales nonnullae.

I consider this specimen to belong to *Grimmia apocarpa* Hedw. on account of the stem which lacks a central strand, of the leaves which are squarrose in all directions when moist, large, broadly ovate-

lanceolate, acute, not concave, strongly keeled, with an entire apex which is either muticous or provided with a very short, toothed, hyaline hairpoint, and on account of the percurrent nerve which is of equal thickness (65 μ) throughout the leaf, smooth at back, plan-convex and of a rather homogeneous cell structure.

The leaf margins are strongly recurved 2–3–stratose and entire. The laminal cells are smooth, rounded or transversely oval, incrassate, and not sinuose at all; in the perichaetial leaves the basal cells are rather hyaline, thin-walled, and elongated.

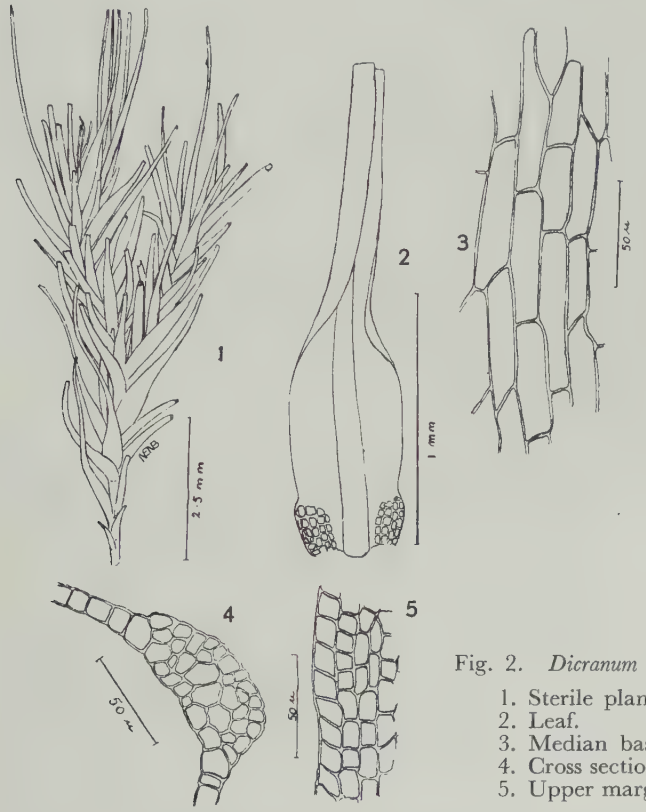

Fig. 2. *Dicranum strictum* Schleich.
1. Sterile plant.
2. Leaf.
3. Median basal leaf cells.
4. Cross section of leaf.
5. Upper marginal leaf cells.

The whole upper half of the leaves has a bistratose lamina and from there many longitudinal bistratose bands extend throughout the lower part reaching nearly to the base. This gives the moss a strange appearance under the microscope, somewhat resembling *Coscinodon cribrosus*, but the bistratose bands scarcely project beyond the leaf surface and the leaves are not plicate. The margin and sometimes the lamina of *Grimmia apocarpa* are described as bistratose, but only at apex and the bistratose bands are not mentioned.

Limpricht (1890–1904, 1: 713) mentions a bistratose lamina for *Schistidium atrofuscum* (Schimp.) Limpr. = *Grimmia apocarpa* var. *atrofusca*

Husnot; he says: "Lamina am Rande und (streckenweise schon oberhalb des Grundes) oberwärts in der ganzen Breite doppelschichtig und schwärzlich". The remark between parentheses (spaced by me) suggests the existence of bands similar to those of the present *Grimmia*. The colour of the moss (blackish) is the same, too. However, the var. *atrofusca* has a distinct central strand, the leaves are erecto-patent when moist, small and subobtuse, concave at the base and with a flat margin in the upper part. Moreover, the habitat and distribution (calcareous rocks in the Alps, above 1800 m altitude) are quite different as well. *Grimmia maritima* has leaves with a bistratose upper part and stems with only a small, indistinct central strand, but the leaves are somewhat contorted when dry, concave and scarcely keeled, whereas the nerve is rough at back, biconvex and provided with two large strands of stereids. Moreover, the leaf apex is not hyaline. So I cannot help but consider the specimen under discussion to represent a new variety, not identical with any of the varieties and species described hitherto. J. J. B.

Orthotrichum lyelli Hook. et Tayl. **var. laeve** Barkman **nov. var.**

Leg. J. J. Barkman no. 2930 (type specimen in Rijksherbarium, Leiden), 22–9–1950, Leens between Zoutkamp and Warffum, H6.**18**.24, prov. of Groningen, on old elm along roadside near village, rather exposed, N. W.–N. E. side of trunk, 88°–91°, 2–3 m high, rel. 518 (*Tortuleto-Ulotetum phyllanthae*).

Differt cellulis foliaribus laevibus.

Though sterile, the specimen may safely be referred to *Orthotrichum lyelli* Hook. et Tayl., on account of the long leaves with brown gemmae on their surface and the flat margins, which are irregularly toothed near apex.

The leaf cells are completely devoid of papillae! J. J. B.

Orthotrichum rivulare Turn.

Leg. J. J. Barkman, no. 3634, 20–7–1951, Spijkenisse, P3.**55**.32, prov. of Z. Holland, on the base of a pollard-willow in low, wet willow-coppice on river-clay along the river Oude Maas. Base of trees flooded by fresh water twice a day. Rel. 974.

In spite of a careful and prolonged search only one stem with about 5 leaves and a few archegonia at apex could be found. The leaves are very typical: broadly ovate-oblong with a rounded apex, recurved margins, small, pellucid, incrassate, irregularly rounded lamina cells with low papillae and a nerve which ceases below the apex.

Since this species was known from Great-Britain, Belgium and Germany, it could be expected here, though it seems to be a more or less mountainous species. It occurs in similar habitats abroad. J. J. B.

Orthotrichum tenellum Bruch **var. decipiens** Vent. **fo. gemmiferum** Barkman **nov. forma**.

Leg. J. J. Barkman, no. 4069 (type specimen in Rijksherbarium, Leiden), 5–7–1951, near Heusden, W. of Nederhemert, Q4.**28**.42,

prov. of Gelderland, on the trunk of a pollard-willow in a meadow, N. side, 62°–81°, 0.5–1.2 m above the soil, rel. 925.

Differt laminis foliorum utrinque copiose gemmiferis; gemmae vel ellipticae, unicellulares, 40μ longae, 34μ latae, vel oblongae usque ad filiformes, subclaviformes, 3–12 cellulares, 60-180μ longae, 29–36μ latae, semper fuscae, cellulis incrassatis.

Gemmae have not yet been reported for this species, as far as I know. On account of the abundant laminal gemmae as well as the rather obtuse leaves with strongly incrassate, rounded cells, one is at first glance inclined to believe that this moss belongs to *Orthotrichum obtusifolium* (which, by the way, was growing mixed with it!), but thanks to the presence of capsules it is quite certain that it belongs to *Orthotrichum tenellum*, which it resembles in all other characters. It belongs to the Central-European var. *decipiens* Vent., which has ribs of 4 rows of cells on the capsule walls. J. J. B.

Pottia lanceolata (Hedw.) C. Müll. var. **gasilieni** (Vent.) Corbière.

Leg. A. J. H. M van de Ven, 1–10–1949, Karstraat, Voerendaal, V6.**23**.12, prov. of Limburg, in grass on cretaceous rocks (Maestrichtien).

The plants were nearly completely buried in the dusty marl. The lower leaves are small, the upper large, oblong and obtuse, the comal leaves still longer (1.6 mm), ovate, acute, spirally twisted when dry, with broadly revolute margins from base to near apex; the nerve is somewhat thickened near apex, with spongious tissue on its ventral face, consisting of thinwalled cells, each with a conical papilla. The lamina cells are smooth. The nerve is excurrent in a rather long, brown hairpoint, 1/9–1/3 of the length of the lamina. Flagellae are sometimes found at the apex of the stem, bearing minute leaves (0.5–0.8 mm long and 0.3–0.4 mm broad).

Venturi (1894) considered this moss to be a species of *Desmatodon* (*D. gasilieni*), Limpricht (1890–1904) called it *Tortula gasilieni*, Moenkemeijer (1927) considered it a variety of *Tortula atrovirens* (Sm.) Lindb. Perhaps this variety deserves specific rank, but at any rate it belongs to the genus *Pottia*, not to the genus *Tortula*. This was already pointed out by Corbière (1895 : 34); his opinion is joined by Thériot and (according to private communications) by Gaume and Potier de la Varde.

The only localities, where it has been found, are Constantine (Algery) and a few places on the W. coast of N. France (peninsula of Cherbourg, Le Havre, Boulogne), on maritime calcareous sand!

My thanks are due to Mr. Gaume (Paris) and Mr. Potier de la Varde (Lez-Eaux par St. Pair/mer) for their valuable information on the taxonomy and distribution. J. J. B.

Seligeria calcarea Br. et Schimp.

Leg. A. J. H. M. van de Ven, 1–10–1949, Wahlwiller, V6.**33**.42, prov. of Limburg, in a *Mesobrometum* on calcareous soil.

The species was detected in a sample mainly consisting of other

mosses. Only five plants were present, on a small piece of marl. Four of them had young sporogons. The gametophytes therefore have to be considered adult; yet they were only 0.75 mm high. Each consisted of a rosette of leaves (slightly more than 0.5 mm long) and a tuft of white rhizoids at the base of the rosette. The whole appearance of the moss is like that of a minute *Isoetes* plant; this impression is also due to the dark green, rigid, succulent and erecto-patent leaves. The succulent texture is caused by the thick nerve occupying the whole breadth of the upper leave part and ending in an obtuse point.

It may be remarked here that DIXON (1924) has not included the genus *Seligeria* in his keys based on gametophyte characters.

Moreover, the sporogons of our plants were too young to yield useful characters. Consequently, when using DIXON for the identification it was not possible to arrive at a satisfying result. I will therefore give supplementary notes to some of his keys here.

Table V (p. XLV) no. 10 has to be changed as follows:

10*a*.	Ls. usually lanceolate	11	
b.	Ls. usually ovate	78	*Bryum*
11*a*.	Nerve reaching the subulate apex; ls. very small	13	*Seligeria*
b.	Nerve not reaching apex; apex not subulate, acute or subacute only	11′	
11′	see no. 11 of DIXON.		

In the same table no. 18 has to be changed as follows:

18*a*.	Cells small, rounded	49	*Zygodon*
b.	Cells more or less quadrate or oblong	18′	
18′*a*.	Stem tall (at least 1 inch); leaf margin entire at base	70	*Bartramia*
b.	Stem very short (less than 2 lines); ls. finely denticulate all round	13	*Seligeria*

Finally, no. 23 also has to undergo a change:

23*a*.	Ls. short, scarcely ½ line long	23′	
b.	Ls. longer, usually over 1 line long	24	
23′*a*.	Nerve occupying the whole subulate upper part of the leaf	13	*Seligeria*
b.	Lamina distinct throughout	49	*Zygodon*

The species was growing mixed with *Camptothecium lutescens*, *Pseudoscleropodium purum*, *Cylindrothecium concinnum*, *Abietinella abietina*, *Fissidens adiantoides*, *Rhytidiadelphus triquetrus*, *Calliergonella cuspidata*, *Ctenidium molluscum*, *Campylium chrysophyllum*, *Thuidium philiberti*, *Amblystegium serpens*, *Mnium rostratum*, *Bryum capillare*, *Oxyrrhynchium praelongum*, *Brachythecium glareosum*, *Barbula unguiculata* and the lichen *Cladonia pyxidata*, all in one sample!

Seligeria calcarea is, as its name suggests, a typical calciphilous moss. On account of its minute size (it is probably the smallest moss of our flora) it is very inconspicuous. To a certain extent this is outweighed by the fact that it usually occurs in great masses. The species was described as long ago as 1790 (by DICKSON) and as it is easy to distinguish from its allies, we meet it in most of the European moss flora's, the very old ones included, which proves that its existence was known to

many students. Therefore a reliable map of its distribution can be based on these flora's (fig. 3). From this map it will be evident that the area is a most interesting one: restricted to N. W. Europe with a centre of dispersal on both sides of the Channel. Beyond this centre the species is extremely rare, for instance in Germany of which the moss flora has been thoroughly investigated. J. J. B.

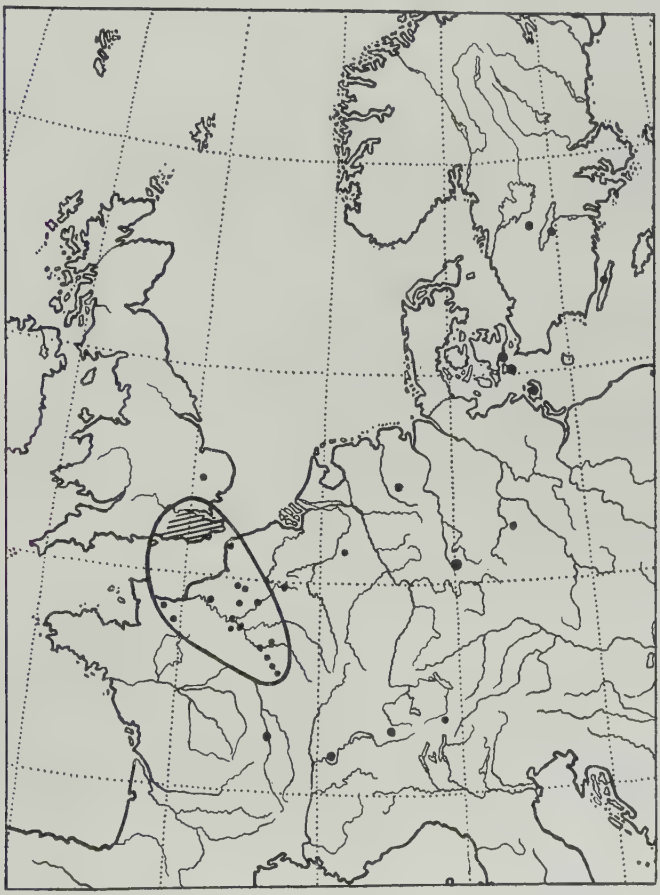

Fig. 3. Distribution of *Seligeria calcarea* Br. et Schimp.

Tortella inclinata (Hedw. fil.) Limpr.

Leg. A. J. H. M. VAN DE VEN, 1–10–1949, Kunraderberg, Heerlen, V6.**23**.22, prov. of Limburg, in a *Mesobrometum*.

The specimens grew in large flat dense mats of a dirty dark olive-brown colour. The plants were only 1–1.5 cm high, the leaves not tortuose when dry, only rather strongly incurved; when moist, they were patent, but incurved at apex. A stem epidermis was present. The central strand was lacking, but, according to DIXON (1924), this

character is not fully reliable if used to distinguish *Tortella inclinata* from *T. flavovirens*. I therefore give this record with some hesitation. The colour of the moss, as well as its stem anatomy, however makes it probable that it belongs to *Tortella inclinata*. I have also seen material of *T. flavovirens* from S. Limburg. This showed the yellow-green colour and much tortuose leaves, typical of that species, and was quite different in appearance. It may be noticed that WACHTER (in JANSEN & WACHTER, 1943), regarded *T. inclinata* as a synonym of *T. flavovirens*, but in this opinion he is supported by very few bryologists.

J. J. B.

Tortula guepinii (Br. et Schimp.) Limpr.

Leg. J. J. BARKMAN, no. 2802, 18–9–1949, St. Pietersberg, Maastricht, prov. of Limburg, in chalk grassland (*Mesobrometum*).

Up till now this species had only been found in France (5 localities) and California. For further details I may refer to BARKMAN (1953).

J. J. B.

Trichostomum crispulum Bruch.

Leg. W. MEIJER, June 1946, Gerendal, prov. of Limburg, on a dry calcareous slope (det. E. A. and W. M.); leg. A. J. H. M. VAN DE VEN: 2–10–1949, Gerendal, V6.**22**.41, prov. of Limburg, in a *Mesobrometum*; 30–9–1949, Schiepersberg between Bemelen and Houthem, V6.**21**.23, prov. of Limburg, on rocks of cretaceous limestone (Maestrichtien) in a *Mesobrometum* (both det. J. J. B.); leg. N. E. NANNENGA-BREMEKAMP, no. 1055, 4–4–1949, Geulem, prov. of Limburg, limestone, with *Ditrichum flexicaule* (det. N. E. N.-B.)

This species is characterised by the narrow, tapering, entire and shortly mucronate leaves with cucullate apices. It is not surprising to find it in our calcareous district: it is a calciphilous moss, mainly occurring in S. Europe, but extending through Central Europe as far as Great Britain, Norway and Sweden. One of us (J. J. B.) found the species near Bettyhill (N. coast of Scotland), in moist dune valleys of calcareous sand mixed with much gravel, immediately behind the beach. It is therefore somewhat surprising that the moss has never been found in the calcareous dunes of Holland; nor has it been reported from the French and Belgian dunes.

var. brevifolium Br. et Schimp.

Leg. A. J. H. M. VAN DE VEN, 1–10–1949, Overeijs, V6.**34**.11, prov. of Limburg, in a *Mesobrometum* (det. J. J. B.)

J. J. B., W. M. and N. E. N.–B.

Weisia crispata C. Müll.

Leg. A. J. H. M. VAN DE VEN, 1–10–1949, Eyser berg, V6.**33**.21, prov. of Limburg, in a *Mesobrometum*.

Although the material is scarce and sterile, I consider it to belong to this species on account of the leaf shape. The species is sometimes considered as a variety (var. *fallax*) of *Weisia tortilis* (= *Hymenostomum*

tortile). The latter species was already known from our country, but the variety was not. These two closely related species (or subspecies or varieties) clearly prove that *Hymenostomum* cannot be separated from *Weisia* as a genus, not even as a section, for that would mean that the two species belong to different sections which is quite unnatural. See also DIXON (1924) and MOENKEMEIJER (1927).

Weisia crispata had been found in Great Britain and Germany and was to be expected here.

J. J. B.

B. HEPATICAE

Fossombronia pusilla (L.) Dum.

Leg. N. E. NANNENGA-BREMEKAMP, no. 2168, 13–9–1952, Botanical Garden "De Wolf", Haren, prov. of Groningen, at the base of a rockery, on moist ground.

A revision of *Fossombronia pusilla* in the herbarium of the K.N.B.V. by WACHTER resigned all the specimens there to other species because of their spore structure (JANSEN & WACHTER, 1935). Since then there are no records of *Fossombronia pusilla* from the Netherlands.

At the time the above specimen was collected, no sporangia were visible, and only when several had ripened a month later, the plants were examined and proved to belong to *Fossombronia pusilla*: the spores had 17–20 spines on the circumference, and not 25–32 as in *Fossombronia wondraczeki*, the only other *Fossombronia* with ridges not forming meshes on the spores. The inner sporangium wall too, is different, having brown semicircular thickenings on the cell walls. (Fig. 4)

W. D. MARG. and N. E. N.–B.

Leptoscyphus taylori (Hook.) Mitt.

Leg. J. J. BARKMAN, no. 3698, 18–9–1951, Huis "Twikkel", M7.**66**.13, Delden near Hengelo, prov. of Overijsel, on the base of an old birch at the edge of a small marshy pond in a tall, dense spruce wood; rather light, but much sheltered, in a moist atmosphere, together with *Lepidozia reptans* and *Ptilidium pulcherrimum*. Rel. 1450.

This curious specimen differs from both *Leptoscyphus anomalus* and *L. taylori* in the following characters: rhizoids are scarce and lacking in the upper part of the stems which are erect; amphigastria are equally scarce; the stems bear clusters of gemmae at their tips, and the leaf cells are only 24–38 μ. However, a few subulate amphigastria have been found. The leaves are orbicular and trigones are very large, nodulose. Moreover, the apical leaves bear 1–2-celled oval gemmae at their margin. So I consider this to be a *Leptoscyphus*, and more particularly *L. taylori*, since the cuticula of the leaves is verrucose (visible on cross-sections of the leaves) and the gemmae bearing leaves are orbicular, without elongated cells.

Although this species is known from Great-Britain and Central Europe (from the Alps to Scandinavia), its discovery in the Netherlands is rather surprising since it mainly occurs in the mountains.

J. J. B.

Lophozia porphyroleuca (Nees)Schiffn.

Leg. E. AGSTERIBBE, no. 1233 and S. GROENHUIJZEN, 21–9–1951, Mosbeek near Vasse, prov. of Overijsel, on a steep bank, facing north, near a brook (Mosbeek).

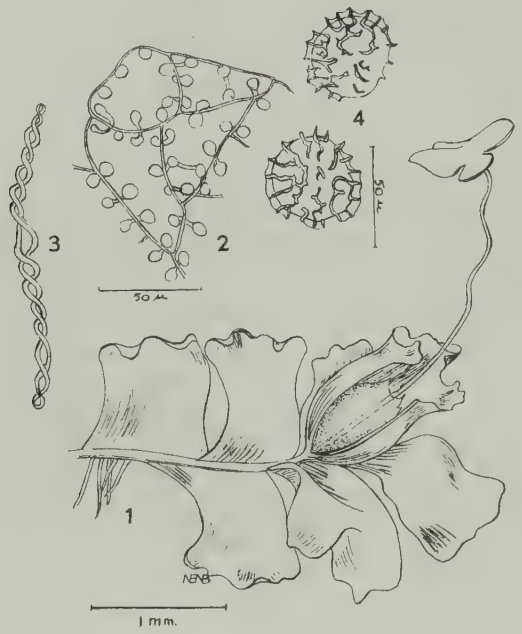

Fig. 4. *Fossombronia pusilla* (L.) Dum. 1. Fertile plant. — 2. Detail of inner sporangium wall. — 3. Elater. — 4. Spores.

The bank on which this hepatic grew was sparsely covered with shrubs of *Vaccinium Vitis-idaea* L. and, being moist and shady, it had a rich liverwort vegetation, a. o. *Lepidozia setacea*, *Nardia geoscypha* and *Calypogeia neesiana*.

Some of the older hepatologists (a.o. K. MÜLLER) considered this hepatic a variety of *Lophozia ventricosa*, but nowadays it is held to be a good species (BUCH, EVANS & VERDOORN, 1938).

In the typical form, as it was encountered here, it is quite different from *Lophozia ventricosa*. The dark reddish-brown colour, reddish leafbase and the leafcells with large trigones will separate it at once from this species. It is usually a plant of the subalpine and alpine regions and appears to be widely distributed all over Europe, though nowhere common. It has a preference for organic substratum, for decaying wood, peat, etc. E. A. and S. G.

Microlejeunea ulicina (Tayl.) Evans

Leg. J. J. BARKMAN, no. 3552, 20–4–1951, Beekhuizen near Velp (Arnhem), P6.**14**.31, prov. of Gelderland, on the base of a rather young beech in a beech wood on acid sandy soil, rel. 706; no. 3557, 21–4–1951,

Middachterbos between Velp and Dieren, P6.**15**.14, prov. of Gelderland, on a 70 years old *Quercus borealis* in open wood (*Querceto-Carpinetum*) on riverclay, N. side of trunk, 72°, 1–2 m high, rel. 721.

This minute atlantic hepatic had not yet been recorded from the Netherlands. Nearest finds: Luxemburg, Normandy, Great-Britain, S. W. Norway. J. J. B.

Pellia neesiana (Gottsche) Limpr. (Fig. 5)

Leg. W. Meijer: 1947–1950, nature reserve Naardermeer, and Kortenhoefse plassen, prov. of N. Holland; Kierse Weide, NW. part of prov. Overijsel; leg. Bryol. Werkgroep, 22–9–1951, Mosbeek near Vasse, prov. of Overijsel.

All these records are from very wet habitats, often with *Phragmites communis* and *Dryopteris thelypteris*. Probably it has often been overlooked.

A key to the indigenous species of *Pellia* follows:

1*a*. Thallus with thickened bands (best seen in longitudinal section). Calyptra exserted; inner wall of capsule with numerous semiannular thickenings 2
b. Thallus without thickened bands. Calyptra included; inner wall of capsule without semiannular thickenings . . . . . *P. endiviaefolia*
2*a*. Margin of involucre absent on the side towards apex of thallus. Paroicous . . . . . *P. epiphylla*
b. Involucre forming a complete ring; mouth erect. Dioicous . . . *P. neesiana*

W. M.

Riccia warnstorfii Limpr.

Leg. W. Meijer, 29–4–1952, Gronsveld, Maastricht, prov. of Limburg, in an abandoned cloverfield, together with *Riccia bifurca;* leg. E. Agsteribbe, no. 1230, 12–9–1952, Botanical Garden "de Wolf", Haren, prov. of Groningen, on a little used path, together with *Riccia beyrichiana* and *R. sorocarpa.*

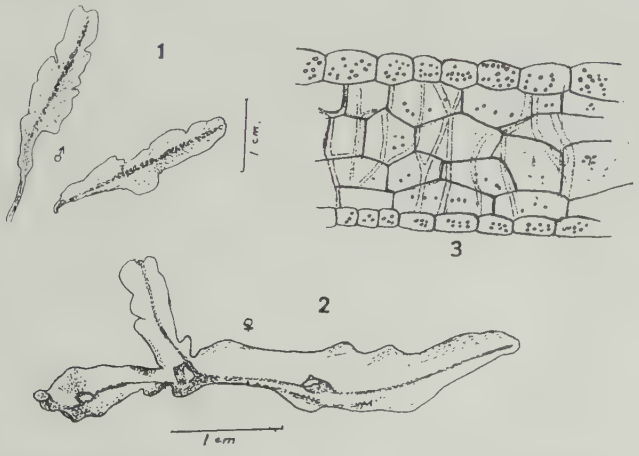

Fig. 5. *Pellia neesiana* (Gottsche) Limpr. 1. Thallus with antheridia. — 2. Thallus with archegonia. — 3. Section of thallus.

Characteristic of this species is the shape of the cross-section of the thallus and the form and branching of the segments. It is closely allied to *R. commutata* but differs by the light-green colour and the obtusely pointed margins of the thallus. According to K. MÜLLER (1916) this hepatic occurs solely north of the Alps.

E. A. and W. M.

II. NEW RECORDS OF RARE AND CRITICAL SPECIES

A. Musci

Amblystegium kochii Br. eur.

Leg. J. J. BARKMAN: no. 3673, 6–5–1951, S. W. of Katwijk, N3.**36**.13, prov. of Z. Holland, on old stems of *Sambucus nigra* in a dense thicket in a wet dune valley, rel. 811; no. 3633, 22–7–1951, Biesbos, Q4.**23**.14, prov. of N. Brabant, on the base of a pollard-willow in a willow coppice, flooded by fresh water twice a day, rel. 998.

These are the second and third localities of the species in the Netherlands. The first record (Zwolle) was by WACHTER from the collection of LAKO (JANSEN & WACHTER, 1940). The two records in the Prodromus (1893) are based upon erroneous determinations.

J. J. B.

Anomodon attenuatus (Schreb.) Hüben.

Leg. J. J. BARKMAN, 21–4–1951, Middachter bos, De Steeg, between Velp and Dieren, P6.**15**.12, prov. of Gelderland, on stumps of *Fraxinus* in low moist ash coppice-wood (*Querceto-Carpinetum filipenduletosum*), rel. 723.

This species appears to be very rare in our country, having been found only near Dordrecht and in a few places in S. Limburg.

J. J. B.

Antitrichia curtipendula (Hedw.) Brid.

Leg. J. J. BARKMAN, no. 3573, 28–4–1951, Speulderbos near Garderen, N5.**18**.13, prov. of Gelderland, on the trunk of an old beech in a beech wood, 1.5–2 m high, W.–NW. side of trunk, 85°–87°, rel. 784, together with *Hypnum cupressiforme var. filiforme* (dominant), *Frullania tamarisci* (subdominant) and *Metzgeria furcata* (abundant).

This is the only recent record for the Netherlands of a species, which is probably nearly extinct now in our country. In the last century it still occurred in 27 localities all over the country!

J. J. B.

Breidleria arcuata (Lindb.) Loeske

Leg. J. J. BARKMAN, no. 3604, 23–4–1951, Reëenberg between Apeldoorn and Elspeet, N6.**12**.11, prov. of Gelderland, along road side in dry oak wood, amongst grasses, in light shade. J. J. B.

Cirriphyllium crassinervium (Tayl.) Loeske

Leg. J. J. Barkman: no. 4126, 19–6–1951, Druten, between Nijmegen and Tiel; P5.**47**.34, prov. of Gelderland; no. 4123, 20–6–1951, Wadenoyen, Geldermalsen, P5.**53**.23, prov. of Gelderland; no. 4072, 5–7–1951, W. of Nederhemert, Heusden, Q4.**28**.42, prov. of Gelderland; no. 3515, 20–4–1952, Tienhoven, P4.**35**.22, prov. of Z. Holland.

All localities are situated in the centre of our country. The specimen from Tienhoven belongs to the var. *turgescens* Mol. (new for the Netherlands), which resembles *Isothecium viviparum* (Neck.) Lindb. very much. Care should be taken not to confound the two mosses when found in sterile state.

All finds mentioned above are on the base of pollard-willows (inundated in winter) in the foreland of our rivers (resp. Waal, Linge, Maas and Lek). The species had not been recorded for our country since 1893; before that time it was known from 5 localities only, three of which were also along rivers, viz. Rotterdam, Kralingen and Zwijndrecht; the other two were situated in the cretaceous district in the extreme S. E. (St. Pietersberg and Geulem), on marl.

J. J. B.

Cryphaea arborea (Huds.) Lindb.

Leg. J. J. Barkman: no. 3695, 16–8–1951, Franeker, H5.**65**.23, prov. of Friesland, on base of old elm tree along road side, rather exposed, rel. 1181; no. 3653, 1–9–1951, de Muy near de Koog, J4.**23**.21, isl. of Texel, prov. of N. Holland, rel. 1240; no. 3651*a*, 22–8–1952, N. of Zandvoort, M3.**38**.12, prov. of N. Holland, rel.1561; no. 3506, 17–5–1952, W. of Noordwijkerhout, M3.**67**.33, prov. of Z. Holland, rel 1511; no. 3511, 30–4–1951, Katwijk, N3.**36**.13, prov. of Z. Holland, rel. 811; no. 3537, 11–5–1952, W. of Wassenaar, N3. **35**.43, prov. of Z. Holland, rel. 1510; no. 3523, 12–4–1952, isl. De Beer, Hoek van Holland, P3.**22**.32, prov. of Z. Holland, rel. 1455; no. 3659, 15–8–1952, isl. of Voorne, P3.**41**.34, prov. of Z. Holland, rel. 1560; no. 3537*a*, 16–4–1952, W. of Ouddorp, Q2.**16**.22, isl. of Goeree, prov. of Z. Holland, rel. 1468; no. 3651, 18–9–1951, Haamstede, Q2.**35**.33, isl. of Schouwen, prov. of Zeeland, rel. 1323; no. 3648, 24–9–1951, N. of Vrouwenpolder, Q2.**63**.34, isl. of Walcheren, prov. of Zeeland, rel. 1362.

It may be remarked that the record of Wachter from Ockenburg S. W. of Den Haag (Jansen & Wachter, 1935) is based on an erroneous determination.

In the previous century this mediterranean-atlantic species was not rare in the Western part of the Netherlands. Nowadays it is extinct nearly everywhere, except in the dunes. The find near Franeker and a find near Nunspeet (Veluwe, prov. of Gelderland) by W. D. Margadant are the only recent records outside the dune area. All recent finds, except Franeker, were on stems of *Sambucus nigra*.

In the dunes I discovered the species in many new localities. This

is probably due to the fact that its very special habitat here had never thoroughly been searched: it always occurs in very dense thickets in valleys near the sea. These thickets are nearly impenetrable. Moreover, though occurring nearly everywhere in the dunes, the species is far from abundant. One often has to examine thoroughly the stems of 50 or 100 shrubs of *Sambucus*, before finding one small tuft of *Cryphaea*.
J. J. B.

Dialytrichia mucronata (Brid.) Limpr.

Leg. W. D. Margadant, 26–8–1945, in a culvert near Kolland, Amerongen, prov. of Gelderland; leg. S. Groenhuijzen: 8–6–1947, Zaltbommel, prov. of Gelderland, on basalt blocks on banks of the Waal; 29–7–1948, near Lexkesveer, opposite Wageningse berg, prov. of Gelderland, in fenland at base of pollard willow; leg. J. J. Barkman: no. 2958, 21–4–1952, Schoonhoven, P4.**34**.41, prov. of Z. Holland, rel. 1498; no. 3532, 20-4-1952, W. of Vianen, P4.**27**.32, prov. of Z. Holland, rel. 1487 and 1488; no. 3603, 19–4–1952, Randwijk S. E. of Wageningen, P5.**38**.12, prov. of Gelderland, rel. 690 and 691; no. 4102, 18-6-1951, Ewijk, W. of Nijmegen, P5.**58**.21, prov. of Gelderland, rel. 880; no. 4022, 23–7–1951, Zevenaar, S. E. of Arnhem, P6.**45**.11, prov. of Gelderland, rel. 1003; no. 3594, 22–4–1951, Rheden, between Velp and Dieren, P6.**15**.31, prov. of Gelderland, rel. 727; no. 3696, 11–10–1951, between Wijhe and Zwolle, M6.**26**.33, prov. of Overijsel, rel. 1398.

If not mentioned otherwise, the habitat of these specimens is on the base of pollard-willows in foreland of rivers, submerged during the winter; so only a few were found on stony substratum. The last two localities are situated along the river IJsel, the others along the Rhine (Lek and Waal). It is remarkable that this mediterranean species was found up to the Dutch-German frontier (Zevenaar), but seems to be lacking in W. Germany. J. J. B., S. G. and W. D. Marg.

Diphyscium foliosum (Hedw.) Mohr

Leg. S. Groenhuijzen and W. D. Margadant, 29–7–1949, hills near Mook, prov. of Limburg.

Fertile specimens were collected on a shady bank of a sunken road. This is, to our knowledge, the first find in our country in this century. The last reference to this species was by W. H. Wachter (1932), who mentions it from the very old collection of J. L. Franquinet.
S. G. and W. D. Marg.

Dolichotheca silesiaca (Selig.) Fleisch.

Leg. J. J. Barkman: no. 4118, 16–6–1951, Watermeerwijk near Berg-en-Dal, Nijmegen, P6.**63**.34, prov. of Gelderland, on the trunk of an old birch in a rather dense, young oak wood, rel. 865; no. 3697, 10–10–1951, Diepenveen, N. of Deventer, M6.**66**.11, prov. of Overijsel, on the base of a young oak in dry brush wood, rel. 1395.

These are the most eastern records of an essentially western species in the Netherlands.
J. J. B.

Fissidens cristatus Wils.

Leg. J. J. Barkman, 21–4–1951, Middachter bos, De Steeg, between Velp and Dieren, P6.**15**.12, prov. of Gelderland, on stumps of *Fraxinus* in low moist ash coppice-wood (*Querceto-Carpinetum filipenduletosum*), rel. 723.

The only Dutch finds hitherto known were situated in S. Limburg (5 localities). J. J. B.

Mnium serratum Schrad.

Leg. R. de Bruin, Rijksherbarium no. 943.235.328, April 1944, Beversluisplaat, Biesbos, prov. of Z. Holland; leg. S. Groenhuijzen, Aug. 1946, Ratumse beek, Winterswijk, prov. of Gelderland; leg. W. D. Margadant, no. 854, 8–4–1950, Grote Valkse beek, Barneveld, prov. of Gelderland, on steep, shaded bank of brook; leg. J. J. Barkman: no. 3636, 3–7–1951, Lage Zwaluwe, Q4.**32**.31, prov. of N. Brabant, rel. 905; no. 3635, 19–7–1951, Heerjansdam, P3.**67**.23, prov. of Z. Holland, rel. 970; 22–7–1951, Biesbos, Q4.**23**.14, prov. of N. Brabant, rel. 998; no. 4035, 26–7–1951, Bekkendelle, Winterswijk, P7.**36**.11, prov. of Gelderland, rel. 1022.

Hitherto *Mnium serratum* had only been found in S. Limburg in the extreme S. E. of our country (calcareous district) on dry, much shaded banks in woods, on marl, and in one locality outside this district: Assinkbrug near Eibergen, prov. of Gelderland, leg. R. van der Wijk, Aug. 1936 (mentioned by Wachter (1937) under the name of *Mnium riparium*, which it is not). The habitat of the localities outside Limburg is a different one, i.e. either on moist banks of brooks or on the base of pollard-willows in dense, low willow-coppice on river clay, flooded by fresh water twice a day. The specimen of De Bruin and those of Barkman, except the last one, were found growing in the last mentioned habitat. Because of the daily submersion the mosses are covered with a thin layer of clay here.

Part of the Limburg specimens and no. J. J. B. 3635 belong to the var. *dioicum* H. Müll. = *Mnium riparium* Mitt. It must be remarked however, that the specimen from Heerjansdam was sterile; it is considered to be the var. *dioicum* on account of the much shorter and broader, ovate leaves and the smaller leaf cells (18–25μ).

J. J. B. and W. D. Marg.

Neckera pumila Hedw.

Leg. J. J. Barkman: no. 2872, 23–9–1950, "Groot Zeewijk", Warffum, G7.**53**.34, prov. of Groningen, on an elm tree at the edge of and protected from sea winds by a park, rel. 529; no. 4048, 5–8–1951, between Zutphen and Doesburgh, N6.**66**.32, prov. of Gelderland, on the base of an old pollard-willow (submerged in winter) in foreland of river IJsel, rel. 1078.

The habitat in both localities is more typical of *Neckera complanata*. Moreover, the leaves of no. 2872 are rather falcate, only partly and indistinctly undulate (both wet and dry) and the tufts are rather

robust and very glossy. Still I consider this specimen to belong to *N. pumila*, since one leaf margin is widely incurved and both are narrowly reflexed. The leaves are only 1.6 mm long, with short cells (1 : 5) and non-porose walls, the larger nerve reaches 1/4 of the leaf length. Flagelliform branches do not occur; instead, the minutely leaved, deciduous ramuli, characteristic of *N. pumila*, are present in the axils of the upper stem leaves. In no. 4048 the leaves are not falcate and more undulate, rather obtuse and crenulate near apex and still shorter (1.2–1.5 mm). Their cells are shorter, too (3–5 : 1), incrassate and non-porose. Only normal branches were observed; these were numerous and short.

The record of Warffum is the northernmost find of this species in the Netherlands, where it is very rare now, mainly occurring on the N. Veluwe (prov. of Gelderland).

var. **philippeana** (B. et S.) Milde

Leg. E. AGSTERIBBE, no. 1232, 30–4–1950, Solse Gat, N. Veluwe, prov. of Gelderland, in a fairly young beech wood on beech, together with *Metzgeria furcata* and *Frullania tamarisci*; leg. J. J. BARKMAN: no. 3601, 25–4–1951, Vierhouter bos, M6.**52**.13, N. Veluwe, prov. of Gelderland, same habitat, rel. 762; no. 3550, 24–4–1951, Elspeter bos, M6.**61**.23, N. Veluwe, prov. of Gelderland, same habitat, rel. 749.

No. 3601 is a mixture of the typical form and the var. *philippeana*, no. 3550 consists of the var. *philippeana* only. E. A. and J. J. B.

Orthodicranum flagellare (Hedw.) Loeske

Leg. W. D. MARGADANT et N. E. NANNENGA-BREMEKAMP, herb. N. E. N.–B. no. 730, 2–4–1950, Oranje Nassau Oord, Wageningen, prov. of Gelderland, in beech wood; leg. N. E. NANNENGA–BREMEKAMP, no. 2383, 4–6-1953, Kievitsdel, Heelsum, prov. of Gelderland, in beechwood.

Our specimens have no flagellae.

W. D. Marg. and N. E. N.–B.

Orthotrichum diaphanum Schrad. **with gemmae**.

Leg. J. J. BARKMAN, no. 2866, 25–9–1950, Delfzijl, H7.**18**.33, prov. of Groningen, on the trunk of an old elm tree in a group of trees, 500 m from the sea coast, rel. 553.

The gemmae are found on the laminae and margins of the leaves and consist of unbranched green filaments of 3–7 cells each.

AMANN & MEYLAN (1912), HUSNOT (1884–1894) and LIMPRICHT (1890–1904) do not mention gemmae at all for this species, BROTHERUS (1923), MOENKEMEYER (1927) and WARNSTORF (1906) include them in their diagnosis of the species. DIXON (1924) suggests them to be restricted to the var. *aquaticum* Davies ex Venturi, but in the original description of that variety (VENTURI, 1873) nothing is said about gemmae and from the description it appears that the var. *aquaticum* was based on quite different characters (broader leaves, shorter hair-

points which are pellucid, but yellowish-green, not hyaline). The specimens with gemmae do not differ in any other features from the species as such. Since gemmae are rare, however, it seemed worth while to mention this find.

It may be remarked, that the key to the species of *Orthotrichum* in DIXON's Handbook needs an emendation both for this form and for the gemmiferous form of *O. tenellum* (see above), as the key leads to *Orthotrichum lyelli* in both cases. J. J. B.

Orthotrichum obtusifolium Schrad.

Leg. J. J. BARKMAN: no. 4070, 5–7–1951, W. of Nederhemert, Heusden, Q4.**28**.42, prov. of Gelderland, on the trunk of a pollard-willow in a meadow, rel. 925; no. 3650, 7–9–1951, Nes, G5.**58**.24, isl. of Ameland, prov. of Friesland, on old elm tree in village, rel. 1304.

In both specimens the stems are very short and turgid with the densely imbricated, short, obtuse leaves. The nerve ends below the apex, the leaf cells are rounded and papillose. Gemmae are abundant on the lamina. For these reasons I consider both specimens to belong to *Orthotrichum obtusifolium*. However, both have leaves with strongly recurved instead of incurved margins! Now, this very character separates the genus *Stroemia* (to which this species belongs) from the genus *Orthotrichum*. I therefore prefer (joining the opinion of DIXON 1924, and MOENKEMEYER, 1927) not to separate the two genera. Perhaps these specimens have to be considered as a new species of *Orthotrichum*, but even then this new species only differs from *Stroemia obtusifolia* (Schrad.) Hagen by the form of the leaf margin. Dr F. OCHSNER (Muri, Switzerland), to whom I sent the no. 3640, wrote to me: "...Was könnte es anders sein als *Orthotrichum obtusifolium*, wie ich es auch aus der Schweiz kenne...Dass es sich um eine besondere Moosform handelt, ist mir klar geworden. Ich würde sie aber, trotz der grösstenteils umgerollten Blattränder, in den Verwandtschaftskreis von *Orth. obtusifolium* stellen. Bei den männlichen Exemplaren waren nämlich die Blattränder grösstenteils flach, wie beim wirklichen *O. obtusifolium*" (the laminae of the leaves are very concave in no. 3640, so that in the male plants the general outline of the leaves, as seen in cross-section, approaches that of genuine *obtusifolium*). Moreover OCHSNER found a few leaves with truly incurved margins and he remarks that the terms "eingerollt" (incurved) and "umgerollt" (recurved) are not clearly distinguished by all authors. MOENKEMEYER (1927) for instance describes the leaves of *O. obtusifolium* as "schwach eingebogen" (p. 626), but in his key (p. 605) as "schwach umgerollt"! The latter term is also used by GAMS (1940) and PICCIOLI says (p. 102): "feuilles . . . peu révolutées sur les bords". Finally OCHSNER remarks: " . . . auf die Art der Blattränder abzustellen, um eine neue Gattung zu kreieren, scheint mir doch etwas gewagt zu sein (Siehe auch die Bemerkung von MOENKEMEYER, 1927, S. 626).". J. J. B.

Orthotrichum pulchellum Brunt.

Leg. J. J. BARKMAN: no. 4034, 26–7–1951, Bekkendelle, Winterswijk,

P7.**36**.11, prov. of Gelderland, on the trunk of an elm tree in moist deciduous forest along a brook (*Querceto-Carpinetum filipenduletosum*), 2–3 m high, rel. 1034; no. 3652, 1–9–1951, de Muy near De Koog, J4.**23**.21, isl. of Texel, prov. of N. Holland, on base of trunk of *Sambucus nigra* in a thicket on top of a low dune ridge near the sea, rel. 1240; no. 3658, 15–8–1952, isl. of Voorne, P3.**41**.34, prov. of Z. Holland, on the extreme base of *Sambucus nigra* in a thicket on the slope of a dune near the sea, rel. 1560. The first of these records was in the *Ulotetum bruchii*, the second and third in the *Cryphaeetum arboreae*.

The Prodromus (1893) cites this species from Helpen, Leeuwarden, Eelderwolde, Utrecht, Maartensdijk and Leiderdorp. Like so many other epiphytic mosses in the Netherlands, this species has become very rare now. It is an atlantic species, occurring in Norway, Denmark, N. W. Germany, the Netherlands, Great-Britain and N. France. In Germany, according to Moenkemeyer (1927), it is rare inland and mainly distributed in the coastal region of the North Sea. J. J. B.

Plagiotheciella latebricola (Wils.) Fleisch.

Leg. W. D. Margadant et N. E. Nannenga-Bremekamp, herb. N. E. N.–B. 731, 2–4–1950, Molenbeekdal, Renkum, prov. of Gelderland, on treestump; leg. J. J. Barkman: no. 3576, 21–4–1951, Middachter bos, De Steeg, between Velp and Dieren, P6.**15**.12, prov. of Gelderland, on decaying wood of an old alder stump in wet alder coppice-wood (*Alnetum glutinosae cardaminetosum amarae*); no. 4109, 14–6–1951, "Het Broek", Hatert, S. W. of Nijmegen, Q6.**11**.24, prov. of Gelderland, on the base of old oaks in dense, tall, shady, moist oak wood (*Querceto-Carpinetum stachyetosum*), rel. 853; no. 3666, 19–6–1951, Bergharen, Druten, between Nijmegen and Tiel, P5.**57**.41, prov. of Gelderland, on the base of a tall oak in oak wood with scattered trees and dense undergrowth of shrubs, on moist sand. Much shaded (N. side of trunk), rel. 888.

J. J. B., W. D. Marg. and N. E. N.–B.

Tortula pulvinata (Jur.) Limpr.

Leg. J. J. Barkman: no. 3639, 4–9–1951, Midsland, H5.**12**.12, isl. of Terschelling, prov. of Friesland, on trunk of elm tree in village, rel. 1272; no. 3644, 7–9–1951, Nes, G5.**58**.24, isl. of Ameland, prov. of Friesland, same habitat, rel. 1303; no. 4105, 18–6–1951, Ewijk, W. of Nijmegen, P5.**58**.21, prov. of Gelderland, on a pollard-willow in foreland of river Waal, rel. 879; no. 4073, 7–7–1951, Beesd, between Leerdam and Geldermalsen, P5.**51**.12, prov. of Gelderland, on a pollard-willow in foreland of river Linge, rel. 944; no. 4172, 2–5–1953, Oostvoorne, P3.**41**.24, prov. of Z. Holland, on trunk of old *Sambucus* in moist dune valley near the sea.

All specimens have a central strand in the stem, an emarginate to obcordate leaf apex, a leaf margin which is only recurved in the lower half (in no. 3644 flat throughout) or only one margin narrowly recurved at base. The leaf cells are rather large (12–15μ). The nerve is nearly smooth at back (except in no. 4172) and forms a long,

hyaline arista, brownish at base. The arista is only slightly rough, except in no. 4172, where it is strongly so. The plants are dioicous. No. 4073 differs somewhat from normal *Tortula pulvinata* by the tall stems, the very long (4–5 mm) and narrow leaves, which are strongly recurved when moist, and the brown hair-points. In no. 4172, too, the leaves are squarrosely recurved when moist, though not strongly. This specimen resembles *Tortula ruralis* in every respect, except the central strand. Obviously the two species are closely related.

All finds hitherto known from the Netherlands (Heer en Cadier, Grave, Maarssen, Kampen) were on stony substratum. J. J. B.

B. Hepaticae

Bazzania trilobata (L.) Gray

Leg. N. E. Nannenga–Bremekamp: no. 662, 22–2–1950, Kievitsdel, Beekdal, Heelsum, prov. of Gelderland; no. 1596, 3–6–1951, Kasteelweg, Beekdal, Heelsum, prov. of Gelderland.

First records outside the hills of Nijmegen. N. E. N.–B.

Lophocolea minor Nees

Leg. J. J. Barkman: no. 3572, 21–4–1951, Middachter bos, De Steeg, between Velp and Dieren, P6.**15**.12, prov. of Gelderland, on stumps of *Fraxinus* in low moist ash coppice-wood (*Querceto-Carpinetum filipenduletosum*), rel. 723; no. 4023, 26–7–1951, "Te Lintum", Winterswijk, P7.**36**.13, prov. of Gelderland, on upper side of fallen, decaying trunk of spruce in young spruce plantation, much sheltered, rather shady, rel. 1017.

Leaves abundantly gemmiferous in both specimens. Remarkably enough, I also observed (in no. 3572) gemmae on a few amphigastria, contrary to the statement of Müller (1916, 1: 811: "Die Unterblätter bleiben von der Gemmenbildung verschont").

In the Netherlands this species only occurs in the calcareous, subcentreurope and fluviatile districts: several finds in S. Limburg (Prodromus Florae Batavae, 1893: 154), one near Venlo (Garjeanne, 1927) and one near Voorst (Zutphen, Gelderland; according to Meyer Drees, 1936). The last mentioned locality and mine are both situated on clay of the river IJsel (fluviatile district).

The Dutch localities constitute the most Western ones of the species area. J. J. B.

Lophozia mildeana (Gottsche) Schiffn.

Leg. W. Meyer, 1951, in a sand-pit, Hargen, Schoorl, prov. of N. Holland, det. H. Persson.

W. H. Wachter first discovered this liverwort for the Netherlands among old herbarium material of the Koninkl. Nederl. Bot. Vereniging (Jansen & Wachter, 1939). This species has a preference for sand-pits. W. M.

Orthocaulis attenuatus (Mart.) Evs.

Leg. C. Engelfriet Jr., herb. W. D. Marg. no. 1172, 4–1952, Posbank, Arnhem, prov. of Gelderland, in tufts of *Leucobryum*; leg. N. E.

NANNENGA-BREMEKAMP: no. 1265, 24–3–1950, Kasteelweg, Beekdal, Heelsum; no. 708, 28–3–1950, Kievitsdel, Beekdal, Heelsum; no. 1378, 30–9–1950, Beek, Nijmegen (all localities in the prov. of Gelderland). W. D. Marg. and N. E. N.–B.

Plectocolea hyalina (Lyell) Mitt.

Leg. E. AGSTERIBBE, no. 1231, 29–4–1951, Trichterberg, V6.**31**.31, prov. of Limburg, on the steep side of a sunken road.

This species prefers loamy roadsides and occurs all over Europe. E. A.

Sphenolobus minutus (Crantz) Steph.

Leg. N. E. NANNENGA-BREMEKAMP: no. 359, 21–2–1950, Kievitsdel, Beekdal, Heelsum; no. 709, 28–3–1950, Kasteelweg, Beekdal, Heelsum (both prov. of Gelderland).

Collected by BUSE in 1854 in practically the same place as I have found it a century later again. N. E. N.–B.

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THE IDENTITY OF MICRANTHUS SERPYLLIFOLIUS ROTH [1]

BY

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(Received Dec. 18th, 1953)

Recently I got the opportunity of examining a specimen from the "Rijksherbarium", Leiden, which was provided with a label on which ROTH had written in the middle the name of the plant, viz. "*Micranthus serpyllifol*-Roth" and in the lower right corner the name of the collector, viz. "Heyne"; in the lower left corner another hand had added "Ind. or. Hb. Roth". As the specimen proved to answer the description of *Micranthus serpyllifolius* given on p. 282 of ROTH's "Novae Plantarum Species, Halberstadt 1821," there can be little doubt that it is either the type of this species or else a duplicate of the latter. This is the more important as none of the authors who in the past ventured an opinion with regard to the taxonomic position of ROTH's species, apparently had seen the type.

ROTH's specimen was inserted in the Leiden Herbarium under the name *Andrographis serpyllifolia* R.W. (*Acanthaceae*), but this is obviously a misidentification, for *Andrographis serpyllifolia* does not fit ROTH's description. The plant described by the latter has smaller and less numerous leaves and its flowers are arranged in terminal spikes instead of solitary or a few together in the axils of ordinary leaves.

It is noteworthy that similar errors have been committed by other botanists. STEUDEL (Nomenclator, ed. 2, 32, 1841) referred ROTH's species to the genus *Aetheilema* R. Br., which is a synonym of *Phaulopsis* Willd., and the Index Kewensi (III, 228, 1894) reduced it to *Phaulopsis parviflora* Willd., which is *Ph. imbricata* (Forsk.) Sweet. According to the description ROTH's plant differed from this species inter alia in the much smaller leaves and spikes, the presence of a single flower in the axil of each bract and of two large bracteoles at the base of each flower; the latter are the "calyx diphyllus" of ROTH's description; his "corolla superne angustata, labio superiore trifido, inferiore integro" is the calyx; the petals, which are very small and inserted on the calyx tube, were overlooked by the author.

The plant described by ROTH is no *Acanthacea*, but represents, as

[1]) This is one of a series of papers based on investigations that were made possible by a grant of the "Nederlandse Organisatie voor Zuiver-Wetenschappelijk Onderzoek (Z.W.O.)".

was already recognized by Nees, who mentions it in his monograph of the *Acanthaceae* (in DC, Prodr. XI, 262, 1847) with the words "omnino non extricanda, sed certe non huius ordinis", an entirely different family. In fact, it proved to belong to the *Lythraceae* (*Lythreae Lythrinae*), and to be identical with the species described by Wight (Icon. I, tab. 257, 1840) under the name *Ameletia tenuis*. The specific epithet of the latter therefore will have to be replaced by that of Roth's species. The position of this species in the genus *Ameletia* DC is somewhat uncertain, but it belongs without doubt to the species that Clarke in Hooker's "Flora of British India" (II, 567, 1879) referred to *Ammannia* L. but which Koehne (in Bot. Jahrb I, 177, 1880) placed, in *Rotala* L. As this seems preferable, I will follow Koehne's example. The correct name for Roth's species therefore becomes **Rotala serpyllifolia** (Roth) Brem. and *Ameletia tenuis* R.W., *Ammannia tenuis* (R.W.) Clarke and *Rotala tenuis* (R.W.) Koehne are reduced to synonyms of the latter.

It is comparatively easy to see how the misconception of Roth's species arose. The trouble started with Steudel, who overlooked the fact that Roth's *Micranthus* was an entirely new genus, and by no means identical with the genus *Micranthus* previously described by Wendland, a description that apparently had escaped Roth's attention. Roth's generic description does not at all agree with that of Wendland's genus, and the fact that Roth gave a generic description and that he added an etymological e.planation of the name, should in itself have been enough to show that an entirely new genus was meant, for when he made use of generic names introduced by other botanists, descriptions and etymological remarks were always omitted. Steudel's mistake was recognized a few years later by Nees (l.c.), but the latter's criticism has apparently always been overlooked; at least neither in the Index Kewensis nor in De Dalla Torre and Harms the genus *Micranthus* Roth is mentioned.

The genus *Micranthus* Wendl. is identical with *Phaulopsis* Willd. and with *Aetheilema* R.Br.; in fact it is the oldest name for this taxon, which is now known as *Phaulopsis*, because botanists of a later period were of opinion that this name ought to be conserved. Steudel's erroneous assumption that *Micranthus* Roth was identical with *Micranthus* Wendl., induced him to transfer Roth's species to the genus *Aetheilema*. He mentions it in his list of species under the name *Aetheilema*? *Rothii* Steud. As stated above, it was recognized already by Nees that Roth's species could not belong to this genus and that it was not even an *Acanthacea*, but as he was unable to assign it its proper place, his remark fell into oblivion, and this explains how the Index Kewensis fifty years later accepted Steudel's reduction as essentially correct; as the genus *Phaulopsis* is apparently represented in India by one species only, viz. the plant known at that time as *Ph. parviflora*, it quite erroneously reduced Roth's species to the latter.

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THE IDENTITY OF SIMIRA TINCTORIA AUBL.

BY

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(Received Dec. 18th, 1953)

AUBLET described and figured in his "Histoire des Plantes de la Guiane Francoise" (Vol. I p. 170–172 and Vol. III t. 65, 1775) under the name *Simira tinctoria* a tree belonging to the family *Rubiaceae* which until very recently was represented in the herbaria solely by specimens that he himself had collected. One of these specimens is preserved in the herbarium of the British Museum (Natural History) and another one in the "Herbier Denaiffe" (cf. LANJOUW, J. and H. UITTIEN in Rec. d. trav. bot. Néerl. *37*, 357, 1940), which was recently acquired by the Muséum d'Histoire Naturelle, Paris.

AUBLET's new genera were viewed in his own time and even long afterwards with considerable distrust, and when we see that their separation from older and already well-known allies is but rarely justified by the contents of his diagnoses, this attitude becomes comprehensible. However, when we take the trouble to examine the material on which his new genera were founded, we are often forced to admit that his intuition had shown him the right way. This applies e.g. to the genera that were separated by him from *Psychotria* L, viz. *Ronabea*, *Tapogomea*, *Carapichea*, *Palicourea*, *Mapouria* and *Nonatelia*. The taxonomists of AUBLET's own time and those of the immediately following period reduced all these genera to *Psychotria*, but these authors based their opinion almost exclusively on AUBLET's insufficiently explicit descriptions. In a later period, when the plants on which AUBLET had founded his genera, were more thoroughly studied, it was gradually recognized that their reduction to *Psychotria* was not justified.

The description of *Simira* is preceded in AUBLET's work by the descriptions of *Ronabea*, *Tapogomoa* and *Carapichea* and followed by those of *Palicourea* and *Mapouria*, all of them genera belonging to the *Psychotrieae* and nearly related to *Psychotria* itself, and the description of *Simira* does not contain a single item that would exclude it from this group. It reads as follows:

"CAL. PERIANTHIUM monophyllum, turbinatum, quinquedentatum
COR. monopetala, tubulosa, disco suprà ovarium inserta; limbus quinquefidus, lobis subrotundis

STAM. FILAMENTA quinque, tubo corollae inserta. ANTHERAE biloculares
PIST. GERMEN ovatum, calice denticulatum, disco coronatum. STYLUS longus, tenuis, apice bipartitus. STIGMATA obtusa
PER. Bacca ovata, calicis dentibus coronata, bilocularis
SEM. solitaria."

Apart from the "lobi subrotundi" of the corolla limb, which suggest, but do not prove, a non-valvate aestivation, there is nothing in this description that does not apply to *Psychotria*. That the ovules are in reality not solitary but densely packed on a placenta with which they form an oblong mass that can easily be mistaken for a solitary ovule, could, of course, not be guessed, and the authors who reduced AUBLET's preceding and following genera to *Psychotria*, had therefore every reason to do the same with *Simira*. The first to treat *Simira* in this way was RAEUSCHEL, who in his "Nomenclator Botanicus" (ed. 3, pl 55, 1797) published the combination *Psychotria tinctoria* [1]. The next was WILLDENOW, who in his "Species Plantarum" (I, 962, 1798) replaced AUBLET's epithet "*tinctoria*" by "*parviflora*". ROEMER and SCHULTES in their "Systema Vegetabilium" (V, 187, 1819) followed suit, but adopting a practice that was not uncommon at that time they used the original generic name "*Simira*" as specific epithet.

The correctness of the view that the genus *Simira* should be reduced to *Psychotria*, has apparently never been questioned, and the fact that the stipules shown in AUBLET's plate are of a type that is never met with either in *Psychotria* or in any of the latter's nearest allies seems to have been completely overlooked. The form of these stipules for a long time puzzled me, but although it made the position of *Simira* in the *Psychotrieae* in my opinion extremely doubtful, it gave no distinct indication with regard to its real position. For this reason I heartily wellcomed the opportunity to study one of AUBLET's specimens, that was offered to me last year during my visit to the British Museum of Natural History. For this opportunity I tender my best thanks to Dr G. TAYLOR, the Keeper of Botany.

It is true that the specimen preserved in the herbarium of the British Museum is a rather poor one, for it does not possess a single complete flower, but it sufficed for the determination of the position of the genus. A cursory examination of the vegetative parts confirmed my surmise that the plant could not belong to the *Psychotrieae*, for raphides proved

[1]) RAEUSCHEL's new combinations can hardly be regarded as validly published (cf. Art. 42 of the International Code of Botanical Nomenclature), for the binomia on which they are based, are omitted, and even the name of the original author is left out. In the case of *Psychotria tinctoria* no mention is made of the basic combination *Simira tinctoria*, and AUBLET's name is not recorded. The main arguments for considering it a new combination based on AUBLET's binomium are that at the time no other *Ps. tinctoria* was known and that Guiana is given as the country of origin. Additional evidence is found in the circumstance that the representatives of AUBLET's other new genera which with more or less right were reduced to *Psychotria*, were treated by him in the same cryptic way. In my opinion, the earlier taxonomists, who ignored RAEUSCHEL's "Nomenclatur" were right.

to be entirely absent. Positive indications as to its position were obtained by a study of the ovary, of which a sufficient number were present. The dissection of one of the latter revealed that AUBLET, as stated above, had made a mistake when he described the ovules as solitary. The placenta, moreover, proved to be of a type that was known so far from one genus only, viz. from *Sickingia* Willd. Everyone who has seen the placentae of this genus, will agree that no mistake is possible (cf. e.g. the figures of the placentae of *S. Glazovii* K. Sch. and of *S. Oliveri* K. Sch. given by SCHUMANN in MARTIUS, Flora Brasiliensis (VI, 6, t. 117 et t. 118, 1889). This similarity in the structure of the placentae would already be sufficient to identify *Simira* with *Sickingia*, but in the other characters too there is a very pleasurable agreement. In *Simira* as well as in *Sickingia* the leaves are large and provided with numerous lateral nerves, the stipules are interpetiolar and long and pointed, raphides are absent and the bast contains a peculiar red dye. AUBLET refers to the latter in the Latin text in the following way: "cortex trunci extùs rufescens, intus rubet" and a little further he adds "cortex utilis ad pannos sericeos et gossipinos rubro colore inficiendos". In the French text he gives more details: "Son écorce est épaisse, roussâtre en dehors et rouge intérieurement" and further "L'écorce de cet arbre trempée dans l'eau lui communique bientôt une couleur d'un beau rouge: on prétend que cette écorce peut être employée dans la teinture. Les essais qu'on en a fait à Caïenne, donnent lieu de croire qu'elle seroit d'une grande utilité pour teindre en rouge vif la soie et le cotton." A similar substance is known to occur in the bast of several *Sickingia* species and may be present in all of them.

The genus *Sickingia* was created by WILLDENOW in "Neue Schriften d. Gesellsch. naturf. Freunde zu Berlin" (III, 445, 1800), and is therefore much younger than *Simira*. It originally comprised two species, viz. *S. erythroxylon* Willd. and *S. longifolia* Willd. SPRENGEL (Syst. Veg. I, 622, 1825) referred *Platycarpum orinocense* M.B. to it, but this was a mistake which was afterwards rectified. Better informed was HOOKER F., who in BENTH. et HOOK. F., Genera Plantarum II, 34, 1873 added *S. cordifolia*. BAILLON, who wrongly reduced *Sickingia* to a subgenus of *Chimarrhis* DC [1], described three more species and transferred a fourth species to it. SCHUMANN in MART., Fl. Bras. VI, 6, 225–234, 1889 restored *Sickingia* to its original rank and raised the number of species to 14, and in the course of this century the latter was brought, mainly by the efforts of STANDLEY, to 32.

In view of the comparatively large number of species that have been described in *Sickingia*, it might seem undesirable to replace this name by *Simira*. It can certainly not been denied that many generic names have been conserved whose claims carried less conviction, but this can hardly be considered a sufficient excuse for a new infringement of the priority rule. In order to make it quite clear that in my opinion all species that have rightfully been referred to *Sickingia* should be

[1] See my remarks on the systematic position of *Sickingia* in Verh. Kon. Ned. Akad. v. Wetensch. Afd. Natuurk. 2e Ser. XLVIII, no 2 (The African Species of Oldenlandia), p. 16, footnote (1952).

transferred to *Simira*, I will, to begin with, do this with the two species on which the genus was founded. For *Sickingia erythroxylon* Willd. I therefore propose the new combination **Simira erythroxylon** (Willd.) Brem. and for *Sickingia longifolia* Willd. the new combination **Simira longifolia** (Willd.) Brem. I am practically certain that all the *Sickingia* species enumerated by SCHUMANN, with the exception of *S. pisoniiformis* (Baill.) K. Sch. which differs i.a. in the very small number of ovules, should be transferred to *Simira*, but I am not fully certain with regard to all the species that subsequently have been described, and for this reason I would prefer to leave the decision to a future monographer. One of the species enumerated by SCHUMANN happens to bear the same epithet as AUBLET's species, but as this *Sickingia tinctoria* (H.B.K.) K. Sch. is conspecific with *Sprucea rubescens* Bth., the epithet of the latter is available for the new combination.

In the introductory paragraph of this paper I stated that *Simira tinctoria* was until very recently known only by AUBLET's specimens. The specimens that recently have come to light, were obtained from a numbered tree in the former Forest Reserve Kaboerie, Suriname and are preserved in the Utrecht Herbarium. Unfortunately the material consists of sterile twigs only.

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ON THE SOFTENING OF FRUITS OF MESPILUS GERMANICA

BY

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(Received Dec. 2nd, 1953)

INTRODUCTION

In 1928 SLOEP (9) came to the conclusion that the rapid physiological softening of the medlar fruit (*Mespilus germanica L.*) is due to an enzyme which apparently can dissolve cell wall pectin (protopectin), but cannot depolymerize nor demethylate dissolved pectin and hence is different from polygalacturonase (PG), depolymerase (DP) and pectin esterase (PE). It is called protopectinase (PP).

Although SLOEP's publication has often been mentioned in literature, her demonstration of a separate PP seems to have been neglected. For instance, KERTESZ in his recent book (4 p. 335) states: "whenever the macerating action of protopectinase can be demonstrated, the enzyme action invariably proceeds to hydrolyze the pectinic acids from protopectin into nonpectic polyuronides and galacturonic acid."

SLOEP's conclusion should have received more attention, for, if true, this means the occurrence of a specific PP at least in one instant.

Therefore we repeated her experiments with the medlar fruit, supplementing them in several respects. Our results contradicted hers in one — rather important — point and this compelled us to reject her conclusion that a specific PP has been demonstrated in the medlar fruit.

The absence of PG and DP in apples and pears had induced KERTESZ (3) to suggest that the softening of ripening fruits might be due to oxydation of cell wall pectin by dehydroascorbic acid or by peroxydes, both of which may arise from oxydative processes with atmospheric oxygen as hydrogen acceptor. SLOEP's experiments were performed in air. With a view on KERTESZ' suggestion, it seemed desirable to repeat them in oxygen-free atmosphere.

EXPERIMENTS AND RESULTS

a. SOFTENING OF FRUIT TISSUE

In the middle of October of the years 1951–1953, fruits were gathered from two trees growing in our botanical garden and stored at 3° C in a refrigerator until used. Many kept hard until December so that each

fall six weeks were available for the experiments. At room temperature the fruits soften or mould much earlier.

First, the following observations of SLOEP were repeated and confirmed. If slices of ripe but hard fruits are held in a chloroform-saturated atmosphere at 30–35° C, they soften within one hour, at the same time showing enzymatic browning. No softening or browning occurs with slices which are not killed, or with slices from fruits, which had been heated either in water of 80° C for 10 min. or in hot air. Neither does softening occur with unripe fruits, gathered in September.

In addition, the following supplementary observations were made.

1. Softening also occurs after killing the fruit tissue by other, not too rigorous means, viz. freezing, vapour of toluene, methanal, acetic acid or ammonia, as well as by a too acid or too alkaline condition, caused by H–, resp. OH-ion-exchanges (see section e).
2. The pH of the press juice is 3,5–4,0 and does not change during softening.
3. Softening also occurs in a nitrogen atmosphere. Fruit slices were put in a vacuum jar and the air was completely replaced by oxygen-free nitrogen, which was accomplished by alternately filling and evacuating. Then, some chloroform was admitted in order to kill the slices. They softened as usual but did not discolour. Evidently, oxygen is not necessary for softening. Hence, it cannot be due to oxydation of cell wall pectin.
4. During softening cell wall pectin is attacked, which is evident from the following experiment.

Ripe fruits were peeled and cut in halves, which were collected in two identical 50 g portions: A and B. Portion A was immediately boiled in ethanol and B after having softened in a chloroform saturated atmosphere for 15 hrs at 30° C. After filtering off the ethanol, both portions were extensively disintegrated either by means of a blendor or by grinding in a mortar with addition of quartz sand until upon microscopical examination, mainly unicellular fragments were found. Then, the samples were analysed for water soluble pectin and, subsequently, acid soluble pectin.

Soluble pectin was extracted from the A and B portions by shaking with 500 ml distilled water at room temperature for 2 hrs. This was repeated three times. Then the residue was centrifuged and extracted 4 times at 80-90° C with 100 ml 0,05 n HCl for 2 hrs with constant stirring. Pectin was determined in the combined water- or acid-extracts, using the Ca-pectate method of Carré and Haynes. The results are given in table 1.

TABLE I

Soluble and insoluble pectin fractions in fresh (A) and softened (B) fruit halves.

Type of pectin	Ca-pectate in % of dry weight of fruit tissue							
	macerated in blendor				ground with sand			
	exp. 1		exp. 2		exp. 3		exp. 4	
	A	B	A	B	A	B	A	B
Water-soluble fraction	5,17	6,90	5,53	6,97	6,07	8,60	4,20	7,47
Acid-soluble fraction .	3,47	2,03	3,40	2,17	3,67	2,30	5,0	2,53
Total	8,64	8,93	8,93	9,14	9,74	10,9	9,2	10,0

Softening causes a small — probably apparent — increase of total pectin and a large increase of the soluble fraction. This is in line with the view that softening is due to solubilization of cell wall pectin, but of course is no proof.

5. This view is confirmed by the observation that Pectasin W, a commercial fungal enzyme preparation containing active pectic enzymes, readily softens the tissue of heated as well as of fresh slices of medlar fruit.

So far, SLOEP's concept of the liberation of an enzyme which attacks cell wall pectin as soon as the parenchyma cells die, may be upheld.

b. ABSENCE OF PECTIN-DEPOLYMERIZATION ACTIVITY

SLOEP inferred the absence of depolymerization-activity from the observation that softened overripe fruit tissue, which had acquired a jelly-like condition as a result of PE-activity, stayed in this condition for a year, when preserved with chloroform. Pieces of Capectate gel, kept in press juice of such fruits, did not change either.

We too observed that gelatinous press juice, preserved with toluene, shows syneresis, but otherwise keeps unchanged for a long time.

In addition, we have tried to detect depolymerization activity by investigating if any galacturonic acid was produced during softening and if the viscosity of a pectin solution decreased after addition of press juice or an extract of fresh fruits or of softened ones.

1. Test for galacturonic acid.

Slices from ripe fruits were halved. One portion of 6 g was ground and extracted with boiling 65% ethanol. The other portion was extracted after having softened in chloroform vapour for 15 hrs at 30° C. The extracts were evaporated and the residues were extracted with 2 ml pyridine at 45° C. This dissolves sugars, uronic acids and uronides, but leaves inorganic salts in the residue. After evaporating the pyridine, the residue was dissolved in water and baker's yeast was added to ferment the sugars. After filtrating and drying, the residue was re-dissolved in a known volume of water. Then, quantities equivalent to 12-125 mg of dry fruit tissue were applied as spots on filter paper and chromatographed (8).

No galacturonic acid or uronides could be detected.

2. Viscometry. We used two methods for determining the viscosity.

Method of DEUEL & WEBER (2). Pectin solution: 4 g low-methoxyl (4%) apple pectin is dissolved in 500 ml water, then 500 ml 0,02 m. Na-oxalate and 50 ml 1 m. acetate buffer solution of pH 4,6 are added. The mixture is heated at 70° C for 1 hr, preserved with toluene and stored for at least one day before use.

Determination. In three 50 ml vials 25 ml. pectin solution of 30° is mixed with 10 ml of the enzyme solution to be tested. Immediately and after ½-4 hrs, 3 m. eq. alkali are added and the total volume is adjusted to 50 ml. After saponification during more than 6 hrs at room temperature, the solution is centrifuged and the flow time is determined at 30° C with a Höppler or an Ostwald viscometer.

Na-pectate method. Because the alkali used for saponification in the previous method, depolymerizes the pectin in proportion of its methoxyl content (10), PE tends to increase the viscosity. To eliminate this effect, we also used a 95% solution of Na-pectate, adjusted to pH 4,5-5,6. The fruit extract was provided with

0,1% sodium-hexa-metaphosphate in order to prevent the formation of Ca-pectate at the moment it was mixed with the pectate solution. In these cases the flow time was determined with an Ostwald viscometer at intervals during the reaction. In a control experiment, heated fruit extract was added.

Using these methods, we have tested juice from ripe hard fruits, from "chloroform-softened" ones and from naturally softened overripe ones. To this end, fruit mash was diluted with an equal volume of water, pressed through cloth and centrifuged.

We also used juice from mashes, which had been adjusted with NaOH to pH 8,0 and to which 0,25 m. NaCl had been added. It is known that under these conditions more PE and DP may be obtained from tomato and other plant materials. On standing, the pH of such a press juice spontaneously decreased to about 6,5 (PE activity). It was used as such and also after re-adjustment of the pH to 4,0.

Furthermore we also tested tannin-free extracts from fresh fruits and softened ones, obtained as follows. The tissue was frozen with solid CO^2, disintegrated, extracted with cold (–20° C) acetone in order to remove tannins and dried. The powder was extracted with water at room temperature or at 3° C, both at its natural pH of 6,5 or at pH 8, either with or without addition of 0,25 m. NaCl. The pH of the extract was kept at 6,7 or adjusted to 4,0.

Since shaking with n-butanol may facilitate enzyme extraction (5), we also used press juice that had been treated with n-butanol, as well as a water-extract of press residue, prepared with a water-butanol mixture. (For these experiments we used fruits that had been stored for several months at -5° C).

Only in one experiment with juice from naturally softened fruits, a small decrease of viscosity was observed. Even if considered significant, this might have been due to an unnoticed mouldy fruit. Although all fruits were carefully scrutinized for occurrence of external or internal mould growth, one is never quite sure, since internal mouldiness is difficult to detect. Mouldiness often occurs in naturally softened fruits. Apparently fungi have an easy access through the top of the fruit, which is the opening of the urn-shaped receptacle.

Apparently, no DP or PG can be obtained from fresh or from physiologically softened medlar fruits by these methods. Usually, these enzymes are typically exo-cellular and may easily be extracted. Therefore it is quite understandable that SLOEP deduced from her experiments that they are absent. In that case, the enzyme responsible for the softening, must be a protopectinase, which is liberated as soon as the protoplasm dies, and is destroyed by a temperature of 80° C.

c. ATTEMPTS TO EXTRACT THE SOFTENING AGENT

According to SLOEP the supposed protopectinase readily diffuses out of softened medlar fruit tissue and can be demonstrated in the juice. She observed maceration of potato-sections and of the collenchyma in transverse sections of *Lamium*-stems, kept in such juice. This did not occur if the juice had been heated. Furthermore, slices

of softened fruits, if put on potato-slices, rapidly softened the latter.

To our astonishment we could not reproduce these observations. Since it was not clear whether SLOEP had used naturally softened overripe fruits, or "chloroform-softened" ones, we tried both, the former after having been scrupulously examined for mouldiness.

Thin potato slices or thicker slices from heated medlar fruits, or several sections (5 μ thick) of *Lamium* stems, were put either between slices from fresh medlar fruits, which were subsequently softened in chloroform vapour, or between slices from naturally softened fruits.

Furthermore, slices of potato or heated medlar fruits as well as *Lamium* sections, were kept in mash, press juice, or extract from fresh, or artificially softened, or naturally softened medlar fruits. The press juices and the extracts were the same as were tested on depolymerization activity (section *b*).

In every case controls were run with heated mash, juice or extract and microbial growth was always prevented by adding chloroform or toluene.

Although most of these experiments were repeated three times, viz. in the fall of the years 1951–1953, never any macerating effect could be observed. However, if overripe *mouldy* fruits were used, maceration did occur. We are compelled to assume that SLOEP had accidently used internally moulded fruits for her experiments on the maceration of *Lamium* and potato tissue, but healthy ones for her experiments which demonstrated the absence of depolymerization.

Here we are confronted with the very remarkable fact that the macerating enzyme apparently does diffuse from the dead protoplasm into the cell wall, but cannot reach tissues which are in contact with the softened tissue and cannot be extracted from softened or unsoftened tissue. Apparently, the enzyme that attacks the cell wall pectin as soon as the cell contents die, is either strongly adsorbed to the cell wall or is inactivated very soon.

In the previous section we reasoned that it must be protopectinase since we failed to demonstrate any depolymerization activity. However, if we likewise fail to obtain the supposed protopectinase *in vitro*, then the macerating enzyme might as well have been a depolymerizing one, PG or DP, differing in one respect from the pectic enzymes known so far, viz. great lability for inactivation by substances likewise occurring in the medlar fruit.

If the enzyme is a protopectinase, then it might merely become strongly adsorbed to the cell wall. This possibility does not apply for a depolymerizing enzyme, for in that case it very probably will continue to depolymerize the pectin to which it is adsorbed and galacturonic acid should have been found in the softened tissue. If it is active for a short time only, this might suffice for solubilization of cell wall pectin, but not for liquefication of pectate gel, neither for production of galacturonic acid in detectable amounts. With mould PG the monomer does not appear until 90 % of the glycosidic bonds have been split (7).

In any case, the softening of the medlar fruit can no more be cited as an example of the occurrence of protopectinase.

d. Attempt to find an inhibitor in medlar fruit tissue

In the acetone-precipitate of pear juice, Weurman (11) has found a thermolabile inhibitor of mould PG, a fact which could be confirmed by us. In addition, he could demonstrate the occurrence of PG or DP-activity in pears of "ideal ripeness".[1] He supposed an inhibition in other stages, but — unfortunately — found that the pear-inhibitor was inactive with the pear-enzyme, which brings doubts as to its role in the softening process of pears.

Since the presence of a strong inhibitor in the medlar fruit might explain our results described in the sections b and c, it still seemed appropriate to find out whether an inhibitor of mould PG also occurs in the medlar fruit.

Therefore, we examined the decrease of viscosity of a mixture of 10 ml 1% Na-pectate, 1 ml 1% Pektasin W enzyme solution and 1 ml extract of medlar fruit tissue. For the blank experiment the extract was heated to the boiling point. The extract was prepared by mixing 4 ml water with 1 g dry medlar fruit powder, obtained by extracting medlar fruit tissue with cold acetone as described in section *b*. The pH of the Na-pectate mixture was adjusted to 5,6 and the decrease of the flow-time was followed at 30° C with an Ostwald viscometer.

No inhibition by medlar fruit extract was observed. Of course, this does not exclude the presence of an inhibitor specific for the macerating enzyme of the medlar fruit.

e. Might the softening be due to ion-exchange?

Plant tissues may be macerated and extraction of pectin may be enhanced by Ca-binding salts, such as polyfosfates and oxalates, as well as by cation-exchangers (1). A similar exchange might be the cause of the liquefication of a soft agar gel by H-saturated Amberlite ion exchanger (6).

Soluble Ca-binding salts cannot be the cause of the softening of the medlar fruit, for in that case the maceration agent should have been easily extractable. However, it is not impossible that killed protoplasm becomes an ion-exchanger as a result of some unknown enzymatic process which is a part of the autolysis which sets in as soon as the cells die. Admittedly, it is not very likely and furthermore it would still be difficult to explain why this ion-exchange process does not occur with *Lamium*-sections suspended in a mash of softened fruits and apparently, is confined to the interaction between the contents and the cell wall of the same cell.

To test this possibility, a layer of synthetic ion-exchange resin was applied between two slices of a medlar fruit that had been heated at 80° C for 10 min. We used Amberlite IRC 50, which has weak acid groups and Amberlite IR 120 as well as Dowex 50, which possess strong acid groups. The resins had been saturated either with acid

[1] Using a Na-pectate solution as substrate and its viscosity as a criterion, we failed to demonstrate depolymerization activity in juice of apples, ripe pears and overripe pears. Neither do these juices macerate *Lamium* sections.

or 5 % NaCl or a 0,5 molar acetate buffer of pH 4. Moreover, the OH-saturated anion-exchangers Ionac A 293 and Dowex 2 were tried.

In no case did softening occur. Softening was evident if the H or OH saturated exchangers were applied on *living* medlar fruit tissue, but evidently, this must have been the result of a killing of the tissue and not of an ion exchange with the cell wall pectin.

Therefore, there is no indication that the softening of the medlar fruit might be due to ion-exchange.

The fact that we performed the experiments described in section *e*, may serve to demonstrate that in our opinion one does not even know with certainty that the softening of the medlar fruit is due to a pectic enzyme, let alone whether this be PP, DP or PG. We only know that in all probability some enzyme system is involved. Since the softening of ripe fruits is a very common and all-important phenomenon, it is to be hoped that the completely negative results of our experiments with the medlar fruit, will not discourage other investigators to pay more attention to the strikingly rapid softening of this fruit, which seems to be an ideal object for investigating the process of softening in ripening fruits in general.

Summary

The claim (9) that the rapid softening of dying tissue of the medlar fruit (*Mespilus germanica* L.) has been proven to be due to protopectinase, has to be cancelled. It can merely be upheld that some enzyme system is involved and that in all probability it is not due to oxydation of pectin, nor to ion-exchange. The softening is accompanied by an increase of soluble and a decrease of insoluble pectin, but no galacturonic acid is produced.

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